

DUAL AMYLIN CALCITONIN RECEPTOR AGONISTS

CROSS REFERENCE TO RELATED APPLICATIONS

This application claims priority to U.S. Provisional Patent Application No.

5 63/618,002 filed on January 5, 2024, the disclosure of which is expressly incorporated herein.

BACKGROUND

Amylin is a 37 amino acid peptide, produced in pancreatic beta cells and co-secreted with insulin. It has important effects on appetite, glucose management, and
10 gastric emptying that warrant investigation for treatment of diabetes and obesity. Human amylin is also involved in cytotoxic amyloid formation in pancreatic islets of Langerhans in patients with type 2 diabetes.

Peptides that constitute full potency agonists at the Amylin (AmyR3) and Calcitonin (CTR) receptors ("DACRA peptides) have demonstrated potent ability to
15 reduce body weight in obese rats and humans. The most advanced drug candidate (cagrilintide) is an analog of the human amylin sequence that has been modified at multiple sites to bestow chemical stability, aqueous solubility and extended duration of action following a weekly administered subcutaneous injection. This drug requires a scheduled dose titration to minimize significant adverse gastrointestinal effects.

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SUMMARY

As disclosed herein, novel peptides exhibiting agonist activity at both the family of amylin receptors and the calcitonin receptor are provided. These novel peptides are referred to herein as DACRA (Dual Amylin Calcitonin Receptor
25 Agonist) peptides. Peptides having both amylin activity and calcitonin activity are particularly advantageous for inducing weight loss or preventing weight gain, as well as for treating metabolic syndrome and diabetes. Thus, in one aspect, the invention provides methods for inducing weight loss or preventing weight gain, which involve administering to a patient in need thereof an effective amount of a peptide, that
30 exhibits activity at the family of amylin receptors and the calcitonin receptor.

The novel DACRA peptides disclosed herein have high balanced potency at all three amylin receptors and the calcitonin receptor where they function as balanced promiscuous agonists to deliver enhanced body weight lowering. The presently disclosed peptide analogs are more potent than cagrilintide, an advanced drug

candidate characterized as a dual amylin/calcitonin co-agonist. Prodrug derivatives, and pharmacokinetic (PK) modifying derivatives thereof, of the novel DACRA peptides disclosed herein are also provided. These prodrug derivatives and PK-modifying derivatives thereof further extend and flatten the pharmacokinetics and pharmacodynamics of these peptides having co-agonists activity at the calcitonin receptor and amylin receptor R3, and optionally at the calcitonin receptor and three amylin receptors.

In accordance with one embodiment a peptide having calcitonin agonist activity is provided, wherein the peptide comprises the sequence of

10 R₄₀-X₁CNTX₅TCAX₉QRLAEFLRH_X₁₉SNNFGX₂₅IL-W₁-X₂₈X₂₉TNVGSNTZ-R₂₀ (SEQ ID NO: 1),

R₄₀-X₁CNTX₅TCATQRLAEFLRHSS-W₁-X₂₈X₂₉TNVGSNTZ-R₂₀ (SEQ ID NO: 6)

or a peptide that differs from SEQ ID NO: 1 or SEQ ID NO: 6 by 1 or 2 amino acid substitutions, optionally wherein the substitutions are conservative amino acid substitutions, wherein

X₁ is Lys, Orn, beta-Orn or beta-Lys, or Lys, Orn, beta-Orn or beta-Lys in a D- stereoisomer configuration;

X₅ is Ala or Ser;

20 X₉ is Thr, isoacyl-Thr, N-acetyl-Thr, or isoacyl-Thr acylated at the alpha amine with a self-cleaving dipeptide disclosed herein;

W₁ is absent or comprises the structure NH₂(CH₂CH₂O)_m(CH₂)_nCOOH, wherein m is an integer selected from the range of 1-20 and n is 1 or 2, optionally wherein m is 2, 4, 6, 8 or 10 and n is 1 or 2, optionally wherein m is 8 and n is 2;

25 X₁₉ is Ser, N-acetyl-Ser, or isoacyl-Ser acylated at the alpha amine with a self-cleaving dipeptide disclosed herein;

X₂₅ is Ala, Pro or hydroxyproline;

X₂₈ is selected from the group consisting of Ser, Orn, Lys, hydroxyproline, N-alpha acyl-ornithine, N-alpha acetyl-Lys, N-acetyl-

30 hydroxyproline, and isoacyl-hydroxyproline acylated at the alpha amine with a self-cleaving dipeptide disclosed herein;

X₂₉ is selected from the group consisting of Ser, Orn, Lys, hydroxyproline, N-alpha acyl-ornithine, N-alpha acetyl-Lys, N-acetyl-

hydroxyproline, isoacyl-Ser acylated at the alpha amine with a self-cleaving dipeptide disclosed herein, and isoacyl-hydroxyproline acylated at the alpha amine with a self-cleaving dipeptide disclosed herein, optionally wherein X_{29} is serine;

5 Z is pipercolic acid, Azetidine-2-carboxylic acid, hydroxyproline or N-acyl-hydroxyproline;

R_{20} is COOH or CONH₂; and

R_{40} is absent, or a C16-C30 fatty acid, a C16-C30 phosphonic fatty acid, a C16-C30 diacid, a C16-C30 alkyl, or a straight chain or branched polyethylene glycol chain having a molecular weight ranging from about 20k to about 40k,
 10 covalently linked to the alpha amine, the beta amine, or the side chain amine of the amino acid at position 1 (X_1), optionally via a first spacer; wherein said first spacer comprises a gamma glutamic acid, a gamma glutamic acid-gamma glutamic acid dipeptide, or a (gamma glutamic acid)_z-[COCH₂(OCH₂CH₂)_k-NH]_q-(gamma glutamic acid)_p, wherein z is 0 or 1, k is an integer selected from the range of 2-4 and q and p
 15 are independently an integer selected from the range of 0-4.

In accordance with one embodiment a peptide having calcitonin agonist activity is provided, wherein the peptide comprises the sequence of

R_{40} - X_1 CNTX₅TCAX₉QRLAEFLRH₁₉SNNFGX₂₅IL-W₁-
 X_{28} X_{29} TNVGSNTZ- R_{20} (SEQ ID NO: 1), or R_{40} - X_1 CNTX₅TCATQRLAEFLRHSS-
 20 W₁- X_{28} X_{29} TNVGSNTZ- R_{20} (SEQ ID NO: 6), wherein

X_1 is Lys, dLys, Orn, beta-Orn or beta-Lys;

X_5 is Ala or Ser;

X_9 is Thr or N-acetyl-Thr;

W₁ comprises the structure NH₂(CH₂CH₂O)_m(CH₂)_nCOOH, wherein m
 25 is an integer selected from the range of 1-20 and n is 1 or 2, optionally wherein m is 2, 4, 6, 8 or 10 and n is 1 or 2, optionally wherein m is 8 and n is 2;

X_{19} is Ser or N-acetyl-Ser;

X_{25} is Ala, Pro or hydroxyproline;

X_{28} is selected from the group consisting of Ser, Orn, Lys,
 30 hydroxyproline, N-alpha acyl-ornithine, N-alpha acetyl-Lys or N-acetyl-hydroxyproline;

X₂₉ is selected from the group consisting of Ser, Orn, Lys, hydroxyproline, N-alpha acyl-ornithine, N-alpha acetyl-Lys or N-acetyl-hydroxyproline, optionally wherein X₂₉ is serine;

Z is pipecolic acid, Azetidine-2-carboxylic acid, hydroxyproline or N-acyl-hydroxyproline;

R₂₀ is COOH; and

R₄₀ is absent, or a C16-C30 fatty acid, a C16-C30 phosphonic fatty acid, a C16-C30 diacid, or a straight chain or branched polyethylene glycol chain having a molecular weight ranging from about 20k to about 40k, covalently linked to the alpha amine, the beta amine, or the side chain amine of the amino acid at position 1 (X₁), optionally via a first spacer; wherein said first spacer comprises a gamma glutamic acid, a gamma glutamic acid-gamma glutamic acid dipeptide, or a (gamma glutamic acid)_z-[COCH₂(OCH₂CH₂)_k-NH]_q-(gamma glutamic acid)_p, wherein z is 0 or 1, k is an integer selected from the range of 2-4 and q and p are independently an integer selected from the range of 0-4. In one embodiment, X₂₅ is Ala, X₂₈ is Orn, Lys, hydroxyproline, N-alpha acyl-ornithine, N-alpha acyl-Lys or N-acyl-hydroxyproline; X₂₉ is Ser, Pro or hydroxyproline; Z is hydroxyproline or N-acyl-hydroxyproline and R₂₀ is CONH₂; and R₄₀ is a C16-C30 phosphonic fatty acid, or a C16-C30 diacid covalently linked to the alpha amine, the beta amine, or the side chain amine of the amino acid at position 1 (X₁), optionally via a spacer comprising gamma glutamic acid.

In one embodiment a peptide of SEQ ID NO: 1 or SEQ ID NO: 6 having amylin/calcitonin co-agonist activity (a DACRA peptide) is provided, wherein R₂₀ is CONH₂ and X₁ is Lys, dLys, Orn, beta-Orn or beta-Lys acylated with a C16-C30 fatty acid, a C16-C30 phosphonic fatty acid, or a C16-C30 diacid, optionally wherein X₁ is beta-Lys, acylated at the beta amine with a C16-C30 diacid, optionally via a spacer. In one embodiment X₁ is Lys, dLys, Orn, beta-Orn or beta-Lys acylated with γE-COC₁₈H₃₆CO₂H, X₂₅ is Ala, Z is 4-hydroxyproline and R₂₀ is CONH₂. In one embodiment X₁ is Lys or beta-Lys that is acylated at the side chain amine with a C16-C30 fatty acid, a C16-C30 phosphonic fatty acid, or a C16-C30 diacid and R₂₀ is CONH₂. In one embodiment X₁ is Lys or beta-Lys that is acylated at the alpha amine of the Lys or the beta amine of beta-Lys with a C16-C30 fatty acid, a C16-C30 phosphonic fatty acid, or a C16-C30 diacid, and R₂₀ is CONH₂. In a further embodiment X₁ is an amino acid in the D-stereoisomer configuration.

In accordance with one embodiment a DACRA peptide, having both amylin and calcitonin agonist activity is provided, wherein the peptide comprises the sequence of

- R_{40} - X_1 CNTX₅TCAX₉QRLAEFLRH₁₉SNNFGX₂₅IL-W₁-
 5 $X_{28}X_{29}$ TNVGSNTZ-R₂₀ (SEQ ID NO: 1), or R_{40} - X_1 CNTX₅TCATQRLAEFLRHSS-W₁- $X_{28}X_{29}$ TNVGSNTZ-R₂₀ (SEQ ID NO: 6), wherein
- R_{40} is absent;
 R_{20} is CONH₂;
 X_1 is Lys, dLys, Orn, beta-Orn or beta-Lys covalently linked via a
 10 second spacer to a straight chain or branched polyethylene glycol chain having a molecular weight ranging from about 20k to about 40k, wherein said second spacer comprises
- i) -[COCH₂(OCH₂CH₂)_k-NH]_q-(alanine-triazole), wherein k is 2, and q is 1 or 2, optionally wherein k is 2, or
 15 ii) -[COCH₂(OCH₂CH₂)_k-NH]_q-(cysteine-S-S), wherein k is 2, and q is 1 or 2, optionally wherein k is 2;
- X_5 is Ala or Ser;
 X_9 is Thr, isoacyl-Thr, N-acetyl-Thr or isoacyl-Thr acylated at the alpha amine with a self-cleaving dipeptide disclosed herein;
 20 W_1 is absent or comprises the structure NH₂(CH₂CH₂O)_m(CH₂)_nCOOH, wherein m is an integer selected from the range of 1-20 and n is 1 or 2, optionally wherein m is 2, 4, 6, 8 or 10 and n is 1 or 2, optionally wherein m is 8 and n is 2;
- X_{19} is Ser, N-acetyl-Ser or isoacyl-Ser acylated at the alpha amine with a self-cleaving dipeptide disclosed herein;
 25 X_{25} is Ala, Pro or hydroxyproline;
 X_{28} is selected from the group consisting of Ser, Orn, Lys, hydroxyproline, N-alpha acyl-ornithine, N-alpha acetyl-Lys, N-acetyl-hydroxyproline and isoacyl-hydroxyproline acylated at the alpha amine with a self-cleaving dipeptide disclosed herein;
 30 X_{29} is selected from the group consisting of Ser, Orn, Lys, hydroxyproline, N-alpha acyl-ornithine, N-alpha acetyl-Lys, N-acetyl-hydroxyproline, isoacyl-Ser acylated at the alpha amine with a self-cleaving dipeptide

disclosed herein, and isoacyl-hydroxyproline acylated at the alpha amine with a self-cleaving dipeptide disclosed herein, optionally wherein X_{29} is serine;

Z is hydroxyproline or N-acyl-hydroxyproline and R_{20} is CONH_2 . In a further embodiment X_1 is Lys or beta-Lys that is covalently linked to a branched

5 polyethylene chain that comprises four branches of 5K each, or four branches of 10K where each branch is linked via a second spacer comprising $-\text{[COCH}_2(\text{OCH}_2\text{CH}_2)_k\text{-NH]}_q\text{-(cysteine-S-S)}$, wherein k is 2, and q is 1 or 2, optionally wherein k is 2;

X_5 is Ala or Ser;

X_9 is Thr or N-acetyl-Thr;

10 W_1 comprises the structure $\text{NH}_2(\text{CH}_2\text{CH}_2\text{O})_m(\text{CH}_2)_n\text{COOH}$, wherein m is an integer selected from the range of 1-20 and n is 1 or 2, optionally wherein m is 2, 4, 6, 8 or 10 and n is 1 or 2, optionally wherein m is 8 and n is 2;

X_{19} is Ser or N-acetyl-Ser;

X_{25} is Ala, Pro or hydroxyproline;

15 X_{28} is selected from the group consisting of Ser, Orn, Lys, hydroxyproline, N-alpha acyl-ornithine, N-alpha acetyl-Lys or N-acetyl-hydroxyproline;

X_{29} is selected from the group consisting of Ser, Orn, Lys, hydroxyproline, N-alpha acyl-ornithine, N-alpha acetyl-Lys or N-acetyl-

20 hydroxyproline, optionally wherein X_{29} is serine;

Z is pipecolic acid, Azetidine-2-carboxylic acid, hydroxyproline or N-acyl-hydroxyproline;

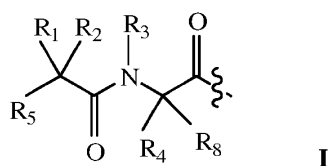
In accordance with one embodiment, prodrug derivatives of any of the amylin analogs, DACRA peptides, calcitonin selective agonists or incretin conjugate

25 derivatives disclosed herein are provided wherein a self-cleaving dipeptide element (A-B) is linked to a primary amine of said peptides disclosed herein where A is an amino acid and B is an N-alkylated amino acid. In one embodiment a peptide of SEQ ID NO: 1 or SEQ ID NO: 6 is provided wherein R_{40} is absent and the dipeptide element (A-B) is linked to the N-terminal alpha amine. In another embodiment a
30 prodrug derivative of any of the incretin conjugates disclosed herein is provided where the dipeptide element (A-B) is linked to the N-terminal alpha amine of the incretin peptide, optionally wherein R_{40} is absent and a second dipeptide element (A-B) is linked to the N-terminal alpha amine. In one embodiment the amylin analogs

disclosed herein are modified to comprise an isoacyl-Thr at amino acid position 9, an isoacyl-Ser at amino acid position 19 or 29, or an isoacyl-hydroxproline at amino acid position 28 or 29 of the peptide, relative to the amino acid sequence of SEQ ID NO: 1, further wherein the alpha amine of said isoacyl-Thr, isoacyl-Ser or isoacyl-hydroxproline is covalently linked via an amide bond to a dipeptide element (A-B), wherein A is an amino acid and B is an N-alkylated amino acid. In one embodiment only one or two of the isoacyl-Thr at amino acid position 9, the isoacyl-Ser at amino acid position 19 or 29, or the isoacyl-hydroxproline at amino acid position 28 or 29 are present in the modified peptides disclosed herein. In one embodiment the self-cleaving dipeptide element (A-B) is linked to the side chain of an amino acid located at position 16, 17, 20, 24, 30 of the incretin peptide component of the incretin conjugate derivatives disclosed herein. In one embodiment the self-cleaving dipeptide element (A-B) is linked to the N-terminal alpha amine DACRA peptide component and/or the incretin peptide component of the incretin conjugate derivatives disclosed herein. In each embodiment comprising the prodrug dipeptide element, the dipeptide can be acylated with fatty acid, phosphonic fatty acid or fatty diacid of sufficient size to bind serum albumin. Alternatively, in one embodiment, the prodrug derivatives of the present invention a PEG polymer can be linked to the dipeptide element wherein the PEG polymer is about 20K to about 40K in size.

In one embodiment a prodrug derivative of any one of the amylin analogs, DACRA peptides, calcitonin selective agonists or incretin conjugate derivatives disclosed herein are provided, wherein the side chain of the first amino acid (A) of the dipeptide element is acylated with a C16-C30 fatty acid, a C16-C30 phosphonic fatty acid, or a C16-C30 diacid, optionally via a dipeptide spacer, wherein said dipeptide spacer comprises a gamma glutamic acid, a gamma glutamic acid-gamma glutamic acid dipeptide, and a $(\text{gamma glutamic acid})_z\text{-[COCH}_2\text{(OCH}_2\text{CH}_2\text{)}_k\text{-NH]}_q\text{-(gamma glutamic acid)}_p$, wherein z is 0 or 1, k is an integer selected from the range of 2-4 and q and p are independently an integer selected from the range of 0-4.

In one embodiment the dipeptide prodrug derivative of the present disclosure comprises the structure:



wherein

(I) R₁ and R₂ are independently selected from the group consisting of H, deuterium, C₁-C₁₈ alkyl, C₂-C₁₈ alkenyl, (C₁-C₁₈ alkyl)OH, (C₁-C₁₈ alkyl)SH, (C₂-C₃ alkyl)SCH₃, (C₁-C₄ alkyl)CONH₂, (C₁-C₄ alkyl)COOH, (C₁-C₄ alkyl)NH₂, (C₁-C₄ alkyl)NHC(NH₂⁺)NH₂, (C₀-C₄ alkyl)(C₃-C₆ cycloalkyl), (C₀-C₄ alkyl)(C₂-C₅ heterocyclic), (C₀-C₄ alkyl)(C₆-C₁₀ aryl)R₇, (C₁-C₄ alkyl)(C₃-C₉ heteroaryl), and C₁-C₁₂ alkyl(W₁₀)C₁-C₁₂ alkyl, wherein W₁₀ is a heteroatom selected from the group consisting of N, S and O, or R₁ and R₂ together with the atoms to which they are attached form a C₃-C₁₂ cycloalkyl;

R₃ is CD₃, C₁-C₁₈ alkyl;

10 R_4 and R_8 are each H or D;

R₅ is NHR₆, or R₅ and R₂ together with the atoms to which they are attached form a 4, 5 or 6 member heterocyclic ring;

R₆ is H or C₁-C₄ alkyl; and,

R₇ is selected from the group consisting of H, OH, halo, (C₁-C₇ alkyl), (C₂-C₇ alkenyl), OCF₃, NO₂, CN, NC, O(C₁-C₇ alkyl), CO₂H, CO₂(C₁-C₇ alkyl), NHR₆, aryl, and heteroaryl;

(II) R₁ is (C₁-C₄ alkyl)NH₂, optionally ILys or dLys, acylated with a C16-
15 C20 fatty acid, a C16-C20 phosphonic fatty acid, or a C16-C20 diacid, optionally via
a dipeptide spacer;

R_2 and R_8 are both H;

R₃ is CD₃, C₁-C₁₈ alkyl;

R₄ is H or R₄ and R₃ together with the atoms to which they are attached form a
20 pyrrolidine, a 3,4-dehydropyrrolidine, a hydroxypyrrolidine, a piperidine or a
hydroxypiperidine ring; and

R₅ is NH₂; or

(III) R_1 is $(C_1-C_4 \text{ alkyl})NH_2$, optionally lLys or dLys, acylated with a C16-C20 fatty acid, a C16-C20 phosphonic fatty acid, or a C16-C20 diacid, optionally via a dipeptide spacer;

R_2 is H;

5 R_4 and R_8 are both D;

R_3 is CD_3 ; and

R_5 is NH_2 ; or

10 (IV) R_1 is $(C_1-C_4 \text{ alkyl})NH_2$, optionally lLys or dLys, acylated with a C16-C20 fatty acid, a C16-C20 phosphonic fatty acid, or a C16-C20 diacid, optionally via a dipeptide spacer;

R_2 , R_4 and R_8 are each H;

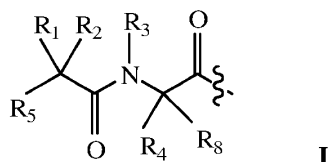
R_3 is CH_3 ; and

R_5 is NH_2 .

15 In one embodiment the side chain of the first amino acid (A) of the prodrug dipeptide element is PEGylated with a straight chain or branched polyethylene glycol chain having a molecular weight ranging from about 20k to about 40k, optionally via a dipeptide spacer comprising the structure:

i) $-[COCH_2(OCH_2CH_2)_k-NH]_q-(\text{alanine-triazole})$, wherein k is 2, and q is 1 or 2, optionally wherein k is 2, or

20 ii) $-[COCH_2(OCH_2CH_2)_k-NH]_q-(\text{cysteine-S-S})$, wherein k is 2, and q is 1 or 2, optionally wherein k is 2. In one embodiment the prodrug dipeptide element comprises the structure:



wherein

25 R_1 comprises a $(C_3-C_4 \text{ alkyl})NH_2$ side chain that has been pegylated, optionally via a dipeptide spacer;

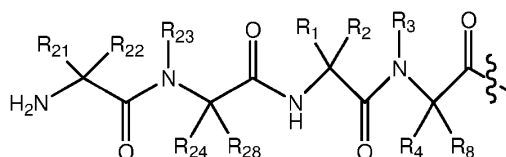
R_2 , R_4 and R_8 are each H;

R_3 is $C_1-C_6 \text{ alkyl}$; and

R_5 is NH_2 , and the dipeptide element spacer is

-[COCH₂(OCH₂CH₂)₂-NH]-(gamma glutamic acid), and the acyl group is a C18-C20 diacid, or a C18-C20 phosphonic fatty acid.

The prodrug dipeptide elements disclosed herein can be further modified by the addition of a second self-cleaving dipeptide to prepare a four amino acid peptide
 5 A-B-C-D referred to herein as a sequential dipeptide. In accordance with one embodiment a prodrug derivative of any of the amylin analogs, DACRA peptides, calcitonin selective agonists or incretin conjugate derivatives disclosed herein is provided further comprising a sequential dipeptide structure of



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covalently linked via an amide bond to a primary amine of the amylin analogs, DACRA peptides, calcitonin selective agonists or incretin conjugate derivatives, wherein

R₁ is (C₁-C₄ alkyl)NH₂ or (C₁-C₄ alkyl)NH₂, linked to a C16-C30 fatty
 15 acid, a C16-C30 phosphonic fatty acid, or a C16-C30 diacid, optionally via a DD1 spacer;

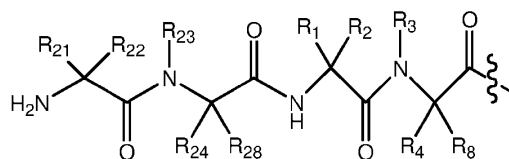
R₂₁ is (C₁-C₄ alkyl)NH₂ or (C₁-C₄ alkyl)NH₂, linked to a C16-C30 fatty
 acid, a C16-C30 phosphonic fatty acid, or a C16-C30 diacid, optionally via a DD2
 spacer, with the proviso that at least one of R₁ or R₂ comprises a C16-C30 fatty acid, a
 20 C16-C30 phosphonic fatty acid, or a C16-C30 diacid;

R₂, and R₂₂ are each H;

R₄, R₈, R₂₄ and R₂₈ are independently H or D;

R₃ and R₂₃ are independently C₁-C₆ alkyl or CD₃, wherein said DD1
 and DD2 prodrug element spacers are independently selected from the group
 25 consisting of a gamma glutamic acid, a gamma glutamic acid-gamma glutamic acid
 dipeptide, and a (gamma glutamic acid)_z-[COCH₂(OCH₂CH₂)_k-NH]_q-(gamma
 glutamic acid)_p, wherein z is 0 or 1, k is an integer selected from the range of 2-4 and
 q and p are independently an integer selected from the range of 0-4.

In one embodiment the sequential dipeptide comprises the structure:



wherein

R₁ is (C₁-C₄ alkyl)NH₂ or (C₁-C₄ alkyl)NH₂, linked to a C16-C30 phosphonic fatty acid, or a C16-C30 diacid via a DD1 spacer;

5 R₂₁ is (C₁-C₄ alkyl)NH₂ or (C₁-C₄ alkyl)NH₂, linked to a C16-C30 phosphonic fatty acid, or a C16-C30 diacid via a DD2 spacer, with the proviso that at least one of R₁ or R₂ comprises a C16-C30 phosphonic fatty acid, or a C16-C30 diacid;

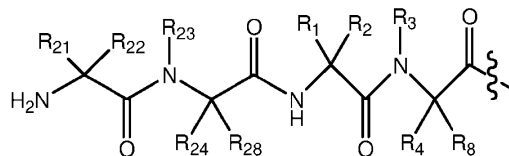
R₂, R₄, R₈, R₂₂, R₂₄ and R₂₈ are each H;

10 R₃ and R₂₃ are independently C₁-C₆ alkyl; and

wherein said DD1 and DD2 spacers are independently

-[COCH₂(OCH₂CH₂)_k-NH]_q-(gamma glutamic acid)_p, wherein k is 2, q is 1 or 2 and p is 0 or 1.

In one embodiment the sequential dipeptide comprises the structure:



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wherein

R₁ is (C₁-C₄ alkyl)NH₂, linked to a C16-C30 phosphonic fatty acid, or a C16-C30 diacid via a DD1 spacer;

20 R₂₁ is (C₁-C₄ alkyl)NH₂ or (C₁-C₄ alkyl)NH₂, linked to a C16-C30 phosphonic fatty acid, or a C16-C30 diacid via a DD2 spacer;

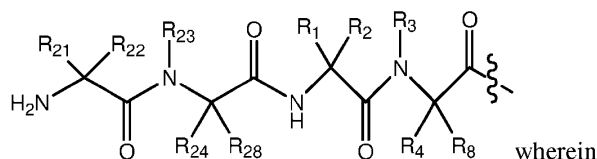
R₂, R₄, R₈, R₂₂, R₂₄ and R₂₈ are each D;

R₃ and R₂₃ are each CD₃;

wherein said DD1 and DD2 spacers are independently

25 -[COCH₂(OCH₂CH₂)_k-NH]_q-(gamma glutamic acid)_p, wherein k is 2, q is 1 or 2 and p is 0 or 1.

In one embodiment the sequential dipeptide comprises the structure of



R₁ comprises a (C₄ alkyl)NH₂ side chain that has been pegylated, via a DD3 spacer, with a straight chain or branched polyethylene glycol chain having a molecular weight ranging from about 20k to about 40k;

5 R₂₁ comprises (C₄ alkyl)NH₂ or a (C₄ alkyl)NH₂ side chain that has been pegylated, via a DD4 spacer, with a straight chain or branched polyethylene glycol chain having a molecular weight ranging from about 20k to about 40k;

R₂, and R₂₂ are independently H, or C₁-C₄ alkyl;

R₄, R₈, R₂₄ and R₂₈ are independently H, D, or C₁-C₄ alkyl;

10 R₃ is CD₃, C₁-C₆ alkyl, or R₃ and R₄ together with the atoms to which they are attached form a substituted or unsubstituted pyrrolidine, hydroxypyridine or piperidine ring; and

R₂₃ is CD₃, C₁-C₆ alkyl, or R₂₃ and R₂₄ together with the atoms to which they are attached form a substituted or unsubstituted pyrrolidine, hydroxypyridine or piperidine ring, wherein said DD3 and DD4 spacers are
15 independently

-[COCH₂(OCH₂CH₂)_k-NH]_q-(alanine-triazole), wherein k is 2, and q is 1 or 2, optionally wherein k is 2, or -[COCH₂(OCH₂CH₂)_k-NH]_q-(cysteine-S-S), wherein k is 2, and q is 1 or 2, optionally wherein k is 2.

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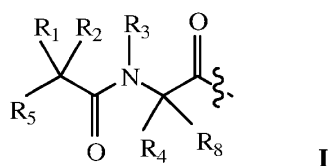
In accordance with one embodiment, a prodrug isoacyl peptide derivative of the DACRA peptides disclosed herein is provided. The combined action of the prodrug and its conversion to the drug yields an extended action, high potency co-agonist where the plasma concentration is tightly controlled to maximize body weight
25 reduction with much lessened risk of GI toxicity. In one embodiment, the prodrug comprises an ester bond between any two adjacent amino acids of SEQ ID NO: 1, with the proviso that when an ester bond is formed between two amino acids, then the second of the two amino acids is alpha amine acylated to stabilize the ester bond against O to N conversion of the ester to the amide, optionally acylated with a moiety
30 comprising a self cleaving dipeptide element. In one embodiment the O to N acyl shift restoring the amide bond is prevented from undergoing conversion to an amide bond

by the covalent linkage of an acylated self-cleaving dipeptide prodrug element linked via to the alpha amine of a hydroxylated amino acid, including for example threonine, hydroxyproline or serine.

In one embodiment, any of the DACRA peptides disclosed herein is further modified, to produce a peptide comprising an isoacyl-Thr at position 9 of the peptide, relative to the amino acid sequence of SEQ ID NO: 1, thus introducing an ester bond between the amino acids at position 8 and 9. Such isoacyl peptide derivatives have been discovered to be inactive at the amylin and calcitonin receptors. A subsequent O to N acyl shift restoring the amide bond between amino acids at positions 8 and 9 restores activity to the DACRA peptide. The timing of the O to N acyl shift can be regulated by the attachment of a self-cleaving dipeptide prodrug element linked to the alpha amine of the isoacyl amino acid. The presence of the dipeptide prodrug element prevents the O to N acyl shift, and subsequent cleavage of the dipeptide element allows the O to N acyl shift to proceed and restore amylin/calcitonin receptor activity to the peptide. The speed of the chemical cleavage of the dipeptide prodrug element is a function of the specific dipeptide prodrug element sequence. Similarly an isoacyl-Ser can be substituted for the amino acid at position 19 or 29 relative to SEQ ID NO: 1, or an isoacyl-hydroxyproline can be substituted for the amino acid at position 28 or 29 relative to SEQ ID NO: 1, wherein a self-cleaving dipeptide prodrug element is linked to the alpha amine of the isoacyl amino acid.

In one embodiment the alpha amine of an isoacyl-Thr located at position 9, or an isoacyl-Ser located at position 19 or 29, or an isoacyl-hydroxyproline located at position 28 or 29 of the amylin analog peptides of the present invention is covalently linked via an amide bond to a dipeptide element (A-B), wherein A is an amino acid, and B is an N-alkylated amino acid. The rate at which the dipeptide prodrug element will chemically cleave is determined based on the side chains of A and B.

In one embodiment an isoacyl peptide derivative of any of the amylin analogs disclosed herein is provided wherein an isoacyl-Thr is present at position 9, or an isoacyl-Ser located at position 19 or 29, or an isoacyl-hydroxyproline located at position 28 or 29, all relative to the sequence of SEQ ID NO: 1 and said isoacyl amino acid is further modified by the covalent linkage of a dipeptide element (A-B) to the alpha amine of the isoacyl amino acid, wherein the dipeptide element comprises the structure:



wherein

R₁ comprises a side chain of (C₁-C₄ alkyl)NH₂, optionally wherein a C₁₆-C₃₀ fatty acid, a C₁₆-C₃₀ phosphonic fatty acid, or a C₁₆-C₃₀ diacid is covalently linked to said side chain, optionally via a dipeptide element spacer;

R₂ is H, or C₁-C₄ alkyl;

R₄ and R₈ are independently H, D, C₁-C₄ alkenyl, C₁-C₄ alkyl, - (CH₂)_nCH₂OH or (C₁-C₄ alkyl)phenyl, wherein n is an integer selected from 0-2;

R₃ is CD₃, C₁-C₆ alkyl, or R₃ and R₄ together with the atoms to which they are attached form a pyrrolidine, 3,4-dehydropyrrolidine, a hydroxypyrrolidine, a piperidine or a hydroxypiperidine ring; and

R₅ is NH₂, wherein said dipeptide element spacer is selected from the group consisting of a gamma glutamic acid, a gamma glutamic acid-gamma glutamic acid dipeptide, and a (gamma glutamic acid)_z-[COCH₂(OCH₂CH₂)_k-NH]_q-(gamma glutamic acid)_p, wherein z is 0 or 1, k is an integer selected from the range of 2-4 and q and p are independently an integer selected from the range of 0-4.

In one embodiment a calcitonin selective analog of amylin is provided, wherein the peptide comprises the sequence of

X₁CNTATCATQRLAEFLRHSSNNFGAIL-R₂₀ (SEQ ID NO: 5) or
X₁CNTATCATQRLAEFLRHSSNNFGAILZSTNVGSN-COOH (SEQ ID NO: 2) or a peptide that differs from SEQ ID NO: 2 or SEQ ID NO: 5 by 1, 2, 3, 4 or 5 amino acid substitutions, optionally wherein the substitutions are conservative amino acid substitutions

wherein

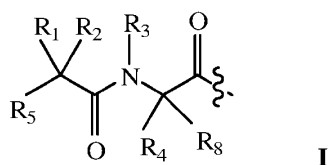
X₁ is Lys, Orn, beta-Orn, or beta-Lys;

Z is hydroxyproline or N-acyl-hydroxyproline; and

R₂₀ is COOH or CONH₂.

In one embodiment a prodrug derivative of a calcitonin selective analog of amylin disclosed herein is provided wherein the prodrug derivative is a isoacyl peptide comprising an ester linked Thr at position 9 of SEQ ID NO: 2 or SEQ ID NO: 5, optionally wherein said isoacyl-Thr further comprises a dipeptide element (A-B)

linked to the alpha amine of the isoacyl-Thr, wherein A is an amino acid and B is an N-alkylated amino acid. In one embodiment a prodrug derivative of a calcitonin selective analog of amylin disclosed herein is provided, wherein the prodrug derivative is an isoacyl protein comprising an ester linked Thr at position 9 of SEQ ID NO: 2 or SEQ ID NO: 5, and a dipeptide element (A-B) linked to the alpha amine of the isoacyl-Thr, wherein the dipeptide has the structure:



wherein

- R₁ comprises a side chain of (C₁-C₄ alkyl)NH₂, optionally wherein a C₁₆-C₃₀ fatty acid, a C₁₆-C₃₀ phosphonic fatty acid, or a C₁₆-C₃₀ diacid is covalently linked to said side chain, optionally via a dipeptide element spacer;
- R₂ is H, or C₁-C₄ alkyl;
- R₄ and R₈ are independently H, D, C₁-C₄ alkyl, C₁-C₄ alkenyl, - (CH₂)_nCH₂OH or (C₁-C₄ alkyl)phenyl, wherein n is an integer selected from 0-2;
- R₃ is CD₃, C₁-C₆ alkyl, or R₃ and R₄ together with the atoms to which they are attached form a pyrrolidine, a 3,4-dehydropyrrolidine, a hydroxypyrrolidine, a piperidine or a hydroxypiperidine ring; and
- R₅ is NH₂, wherein said dipeptide element spacer is selected from the group consisting of a gamma glutamic acid, a gamma glutamic acid-gamma glutamic acid dipeptide, and a (gamma glutamic acid)_z-[COCH₂(OCH₂CH₂)_k-NH]_q-(gamma glutamic acid)_p, wherein z is 0 or 1, k is an integer selected from the range of 2-4 and q and p are independently an integer selected from the range of 0-4.

In one embodiment a conjugate of any of the DACRA peptides disclosed herein is provided wherein the amylin analog is conjugated to an incretin peptide that exhibits agonist activity at any one of the glucagon, GIP and GLP-1 receptors, or co-agonist activity at the GIP and GLP-1 receptors, or exhibits tri-agonist activity at the glucagon, GIP and GLP-1 receptors. The incretin peptide can be any of the known incretins including for example native glucagon, GLP-1, or GIP, or an agonist analog of glucagon, GLP-1, or GIP, including a glucagon/GLP-1 co-agonist, a GIP/GLP-1 co-agonist, and a glucagon/GIP/GLP-1 tri-agonist. In one embodiment the incretin

component of the DACRA conjugate is selected from Semaglutide (SEQ ID NO: 10), tirzepatide (SEQ ID NO: 11), retatrutide (SEQ ID NO: 12) and analogs thereof. In one embodiment the incretin component of the DACRA conjugate comprises the structure of

5 YX₂X₃GTX₆X₇SDYSIX₁₃LX₁₅KIAQX₂₀AFVQWLIAGGPSSGAPPPS-

R₂₀ (SEQ ID NO: 14) or

YX₂X₃GTFTSDYSIX₁₃LX₁₅KX₁₇AQX₄₀AFVQWLLEGGPSSGAPPPS-

R₂₀ (SEQ ID NO: 15)

wherein

10 X₂ is Aib;

X₃ is Glu or Gln;

X₆ is alpha methylated Phe or Phe;

X₇ is isoacyl-Thr, Thr, or isoacyl-Thr acylated at the alpha amine with a self-cleaving dipeptide disclosed herein dipeptide element (A-B), wherein A is

15 an amino acid and B is an N-alkylated amino acid;

X₁₃ is Aib or alpha methylated Leu;

X₁₅ is Glu or Asp;

X₄₀ is Aib;

R₂₀ is CONH₂; and

20 X₁₇ and X₂₀ are independently an amino acid comprising a (C1-C4 alkyl)NH₂ side chain that has been acylated with a C16-C20 acyl group, a C16-C20 phosphonic fatty acid, or a C16-C20 alkyl group, optionally via an conjugate spacer; wherein the conjugate spacer comprises the structure: -[COCH₂(OCH₂CH₂)_kNH]_q- (gamma glutamic acid)_p-; wherein k is 2, p is 1 or 2 and q is an integer selected from

25 1, 2 or 4, optionally wherein k is 2, p is 1 and q is 2. In one embodiment the incretin peptide comprises the sequence of SEQ ID NO: 14 wherein X₂ is Aib, X₃ is Glu, X₆ is alpha methylated Phe, X₇ is said acylated isoacyl-Thr or Thr; X₁₃ is Aib, X₁₅ is Glu, and X₂₀ is Lys acylated via its side chain with (COCH₂(OCH₂CH₂)₂NH)₂-γE-

30 γE-COC₁₈H₃₆CO₂H, or Lys acylated via its side chain with (COCH₂(OCH₂CH₂)₂NH)₂-γE-COC₁₈H₃₆PO₃H₂ and R₂₀ is CONH₂. In one embodiment the incretin peptide

comprises the sequence of SEQ ID NO: 15 wherein X₂ is Aib, X₃ is Gln, X₁₃ is alpha methylated Leu, and X₁₅ is Asp; X₁₇ is Lys acylated via its side chain with (COCH₂(OCH₂CH₂)₂NH)₂-γE-COC₁₈H₃₆CO₂H or Lys acylated via its side chain with (COCH₂(OCH₂CH₂)₂NH)₂-γE-COC₁₈H₃₆PO₃H₂, X₄₀ is Aib and R₂₀ is CONH₂.

In one embodiment a DACRA peptide is provided comprising the structure of
 $R_{40}-X_1\text{CNTX}_5\text{TCATQRLAEFLRHSSNNFGX}_{25}\text{IL}-W_1X_{28}X_{29}\text{TNVGSNTZ-CONH}_2$ (SEQ ID NO: 9), $R_{40}-X_1\text{CNTATCATQRLAEFLRHSSNNFGAIL}-W_1-$
 $X_{28}\text{STNVGSNTZ-CONH}_2$ (SEQ ID NO: 4), $R_{40}-X_1\text{CNTATCATQRLAEFLRHSS-}$
5 $W_1-X_{28}\text{STNVGSNTZ-CONH}_2$ (SEQ ID NO: 8), or a peptide that differs from SEQ ID NO: 4, SEQ ID NO: 8 or SEQ ID NO: 9 or a peptide that differs from SEQ ID NO: 4, SEQ ID NO: 8 or SEQ ID NO: 9 by 1, 2, 3, 4 or 5 amino acid substitutions, optionally wherein the substitutions are conservative amino acid substitutions, wherein

10 X_1 is Lys, Orn, beta-Orn or beta-Lys;
 X_5 is Ala or Ser;
 X_{25} is Ala, Pro or hydroxyproline;
 X_{28} is Ser, Orn, Lys, hydroxyproline, N-alpha acyl-ornithine, N-alpha acyl-Lys or N-acyl-hydroxyproline;

15 X_{29} is Ser, Pro or hydroxyproline;
Z is hydroxyproline or N-acyl-hydroxyproline; and
 W_1 is a bifunctional linker comprising the structure
 $[\text{NH}_2(\text{CH}_2\text{CH}_2\text{O})_m(\text{CH}_2)_n\text{COOH}]$, wherein m is an integer selected from the range of 1-20 and n is 1 or 2, optionally wherein m is 2, 4, 6, 8 or 10 and n is 1-2, optionally
20 wherein m is 8 and n is 2;

R_{40} is C16-C30 fatty acid, C16-C30 diacid group or a C16-C30 alkyl group covalently linked to the N-terminal alpha amine, the beta amine, or the side chain amine of the amino acid at position 1 (X_1), optionally via a spacer; wherein said spacer is selected from the group consisting of a gamma glutamic acid, gamma
25 glutamic acid-gamma glutamic acid dipeptide, $[\text{NH}-(\text{CH}_2\text{CH}_2\text{O})_m-\text{COCH}_2-]_n$ and a (gamma glutamic acid) $_z$ - $[\text{COCH}_2(\text{OCH}_2\text{CH}_2)_k-\text{NH}]_q$ -(gamma glutamic acid) $_p$, wherein m is an integer selected from the range of 2-4, n is an integer selected from the range of 1-10, z is 0 or 1, k is an integer selected from the range of 2-4 and q and p are independently an integer selected from the range of 0-4, optionally wherein an
30 incretin peptide is linked to the free amine of the amino acid at position 28 (i.e., if the amino acid at position 28 (X_{28}) is linked to W_1 via its alpha amine, then the incretin is linked to the side chain amine of the amino acid at position 28 (X_{28}) and vice versa), optionally via a conjugate spacer, wherein said conjugate spacer comprises the

structure: $[\text{NH}_2(\text{CH}_2\text{CH}_2\text{O})_m(\text{CH}_2)_n\text{COOH}]$, wherein m is an integer selected from the range of 1-20 and n is 1 or 2, optionally wherein m is 2, 4, 6, 8 or 10 and n is 1 or 2.

In one embodiment the conjugate peptide comprises the structure of

$\text{R}_{40}\text{-X}_1\text{CNTATCATQRLAEFLRHSSNNFGAIL-W}_1\text{-X}_{28}\text{STNVGSNTZ-}$

5 CONH_2 (SEQ ID NO: 4), $\text{R}_{40}\text{-X}_1\text{CNTATCATQRLAEFLRHSS-W}_1\text{-X}_{28}\text{STNVGSNTZ-CONH}_2$ (SEQ ID NO: 8), or a peptide that differs from SEQ ID NO: 4 or SEQ ID NO: 8 by 1, 2, 3, 4 or 5 amino acid substitutions, optionally wherein the substitutions are conservative amino acid substitutions wherein

X_1 is Lys, Orn, beta-Orn or beta-Lys;

10 X_{28} is Lys or Orn linked to W_1 via the alpha amine or the side chain amine of the amino acid at position 28;

Z is hydroxyproline;

W_1 is an optional bifunctional linker comprising the

15 $[\text{NH}_2(\text{CH}_2\text{CH}_2\text{O})_m(\text{CH}_2)_n\text{COOH}]$, wherein m is an integer selected from the range of 1-20 and n is 1 or 2, optionally wherein m is 2, 4, 6, 8 or 10 and n is 1 or 2, optionally wherein m is 6, 8 or 10 and n is 1, optionally wherein m is 6, 8 or 10 and n is 2; and

R_{40} is a C16-C30 fatty acid, C16-C30 diacid group or a C16-C30 alkyl group covalently linked to the N-terminal alpha amine, the beta amine, or the side chain amine of the amino acid at position 1 (X_1), optionally via a spacer; wherein said spacer is selected from the group consisting of a gamma glutamic acid, gamma glutamic acid-gamma glutamic acid dipeptide, $[\text{NH}-(\text{CH}_2\text{CH}_2\text{O})_m\text{-COCH}_2\text{-}]_n$ and a (gamma glutamic acid) $_z$ - $[\text{COCH}_2(\text{OCH}_2\text{CH}_2)_k\text{-NH}]_q$ -(gamma glutamic acid) $_p$, wherein m is an integer selected from the range of 2-4, n is an integer selected from the range of 1-10, z is 0 or 1, k is an integer selected from the range of 2-4 and q and p are independently an integer selected from the range of 0-4; and an incretin peptide linked to the free amine of the amino acid at position 28 (i.e., if the amino acid at position 28 (X_{28}) is linked to W_1 via its alpha amine, then the incretin is linked to the side chain amine of the amino acid at position 28 (X_{28}) and vice versa), via a conjugate spacer. In one embodiment the optional conjugate spacer is selected from the group consisting of a gamma glutamic acid, gamma glutamic acid-gamma glutamic acid dipeptide, $[\text{NH}_2(\text{CH}_2\text{CH}_2\text{O})_m(\text{CH}_2)_n\text{COOH}]$ and a (gamma glutamic acid) $_z$ - $[\text{COCH}_2(\text{OCH}_2\text{CH}_2)_k\text{-NH}]_q$ -(gamma glutamic acid) $_p$, wherein m is an integer

selected from the range of 1-20, n is 1 or 2, z is 0 or 1, k is an integer selected from the range of 2-4 and q and p are independently an integer selected from the range of 0-4. In one embodiment the optional conjugate spacer comprises the structure $[\text{NH}_2(\text{CH}_2\text{CH}_2\text{O})_m(\text{CH}_2)_n\text{COOH}]$, wherein m is an integer selected from 2, 4, 6, 8 or 10 and n is 1 or 2, optionally wherein m is 6, 8 or 10 and n is 2. In one embodiment both W_1 and the conjugate linker are independently $\text{NH}_2(\text{CH}_2\text{CH}_2\text{O})_m\text{CH}_2\text{CH}_2\text{COOH}$, wherein m is 6, 8 or 10. In one embodiment the incretin peptide is selected from a commercially available incretin including for example semaglutide, tirzepatide or retatrutide.

10 In accordance with one embodiment the conjugate peptide comprises the structure of

$\text{R}_{40}\text{-X}_1\text{CNTATCATQRLAEFLRHSSNNFGAIL-W}_1\text{-X}_{28}\text{STNVGSNTZ-CONH}_2$ (SEQ ID NO: 4), $\text{R}_{40}\text{-X}_1\text{CNTATCATQRLAEFLRHSS-W}_1\text{-X}_{28}\text{STNVGSNTZ-CONH}_2$ (SEQ ID NO: 8), or a peptide that differs from SEQ ID NO: 4 or SEQ ID NO: 8 by 1, 2, 3, 4 or 5 amino acid substitutions, optionally wherein the substitutions are conservative amino acid substitutions wherein

X_1 is beta-Lys;

X_{28} is Orn or Lys linked to W_1 via the alpha amine or the side chain amine of Orn;

20 Z is hydroxyproline;

W_1 is an optional bifunctional linker comprising the structure $[\text{NH}_2(\text{CH}_2\text{CH}_2\text{O})_m(\text{CH}_2)_n\text{COOH}]$, wherein m is an integer selected from the range of 4-10 and n is 1 or 2, optionally wherein m is 2, 4, 6, 8 or 10 and n is 2, optionally wherein W_1 is $\text{NH}_2(\text{CH}_2\text{CH}_2\text{O})_8\text{CH}_2\text{CH}_2\text{COOH}$; and

25 R_{40} is a C16-C30 fatty acid, C16-C30 diacid group or a C16-C30 alkyl group covalently linked to the beta amine, or the side chain amine of beta-Lys, optionally via a spacer; wherein said spacer is selected from the group consisting of a gamma glutamic acid, gamma glutamic acid-gamma glutamic acid dipeptide, $[\text{NH}_2(\text{CH}_2\text{CH}_2\text{O})_m(\text{CH}_2)_n\text{COOH}]$ and a (gamma glutamic acid)z-

30 $[\text{COCH}_2(\text{OCH}_2\text{CH}_2)_k\text{-NH}]_q\text{-(gamma glutamic acid)}_p$, wherein m is an integer selected from the range of 2-4, n is 1 or 2, z is 0 or 1, k is an integer selected from the range of 2-4 and q and p are independently an integer selected from the range of 0-4, and an incretin peptide linked to

i) the alpha amine of the Orn at position 28, wherein the side chain amine of the Orn at position 28 is covalently linked to W_1 ; or

ii) the side chain amine of the Orn at position 28, wherein the alpha amine of the Orn at position 28 is covalently linked to W_1 , optionally via the conjugate spacer.

5 In one embodiment the conjugate peptide comprises the structure of

R_{40} - X_1 CNTATCATQRLAEFLRHSSNNFGAIL- W_1 - X_{28} STNVGSNTZ-

CONH₂ (SEQ ID NO: 4), or R_{40} - X_1 CNTATCATQRLAEFLRHSS- W_1 -

X_{28} STNVGSNTZ-CONH₂ (SEQ ID NO: 8), wherein

R_{40} is γ E-COC₁₈H₃₆CO₂H or γ E-COC₁₈H₃₆PO₃H₂ linked to the N-terminal

10 alpha amine or beta amine of the amino acid at position 1 of said peptide;

X_1 is Lys or beta-Lys;

W_1 is NH₂(CH₂CH₂O)_mCH₂CH₂COOH, wherein m is 8,

X_{28} is Orn or Lys linked to W_1 via the alpha amine or the side chain amine of Orn or Lys, optionally wherein X_{28} is Lys linked to W_1 via the alpha amine of Lys;

15 and

and an incretin peptide linked to

i) the alpha amine of the Orn or Lys at position 28 via a conjugate spacer, wherein the side chain amine of the Orn or Lys at position 28 is covalently linked to W_1 ; or

20 ii) the side chain amine of the Orn or Lys at position 28 via a conjugate spacer, wherein the alpha amine of the Orn or Lys at position 28 is covalently linked to W_1 ;
or

iii) the side chain amine of the Lys at position 28 via a conjugate spacer, wherein the alpha amine of the Lys at position 28 is covalently linked to W_1 , wherein

25 the conjugate spacer is NH₂(CH₂CH₂O)₈CH₂CH₂COOH.

In accordance with one embodiment a pharmaceutical composition is provided comprising any of the calcitonin selective amylin analogs, the novel DACRA peptides, the incretin/DACRA conjugates or PK modified derivatives and prodrug derivatives thereof, as disclosed herein, preferably at a purity level of at least 90%,
30 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99%, and a pharmaceutically acceptable diluent, carrier or excipient. Such compositions may contain the calcitonin selective amylin analogs, the novel DACRA peptides, the incretin/DACRA conjugates or PK modified derivatives or prodrug derivatives thereof, as disclosed herein, at a concentration of about 2 to about 50 mg/ml, or about 2 to about 20 mg/ml,

or about 5 to about 20 mg/ml or at a concentration of at least 2mg/ml, at least 5 mg/ml or at least 10mg/ml, or any bioequivalent concentration of the foregoing. In one embodiment the pharmaceutical compositions comprise aqueous solutions that are sterilized and optionally stored within various package containers. In one
5 embodiment aqueous formulations are prepared in dimethyl sulfoxide (DMSO), including about 30% to 100% DMSO, about 50% to 100% DMSO, about 70% to 100% DMSO, or about 90% to 100% DMSO, with the remainder water. In one embodiment formulations are prepared in 100% dimethyl sulfoxide. In other embodiments the pharmaceutical compositions comprise a lyophilized powder. The
10 pharmaceutical compositions can be further packaged as part of a kit that includes a disposable device for administering the composition to a patient. The containers or kits may be labeled for storage at ambient room temperature or at refrigerated temperature.

In accordance with one embodiment an improved method of reducing weight
15 gain or inducing weight loss in patients in need thereof while reducing adverse side effects is provided. The method comprises the steps of administering any of the DACRA peptides or the incretin/DACRA conjugates disclosed herein, or prodrug derivatives thereof, in an amount therapeutically effective in reducing weight gain or inducing weight loss in a subject.

20 In accordance with one embodiment a method for increasing bone growth or preventing bone loss in a subject in need thereof is provided. In one embodiment the method comprises administering to said subject a calcitonin selective amylin analog disclosed herein, or prodrug derivative thereof, in an amount therapeutically effective to increase bone growth or decrease bone loss.

25 In accordance with one embodiment an improved method of treating diabetes in patients in need thereof is provided. The method comprises the steps of administering any of the incretin/DACRA conjugates disclosed herein, or prodrug derivatives thereof, in an amount therapeutically effective to lower blood glucose levels and induce weight loss. In one embodiment the conjugate comprises any of the
30 DACRA peptides disclosed herein conjugated to an incretin peptide.

All therapeutic methods, pharmaceutical compositions, kits and other similar embodiments described herein contemplate that the use of the terms peptides, agonists, co-agonists, or peptides includes all pharmaceutically acceptable salts or esters thereof.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 provides the sequences of native amylin (SEQ ID NO: 16); pramlintide (SEQ ID NO: 17), cagrilintide (SEQ ID NO: 18) and amylin analog MBX 5129 (SEQ ID NO: 38), wherein O = (γ E-COC₁₈H₃₆CO₂H), Z = 4-hydroxyproline, X = beta-lysine and X = beta-lysine.

Figs. 2A-2C provide the results of an *in vitro* assay measuring the activity of amylin analogs 5053 (SEQ ID NO: 26), 5061 (SEQ ID NO: 27), 5063 (SEQ ID NO: 28), and 5064 (SEQ ID NO: 29) at the human amylin, hAMY3R (Fig. 2A) or human calcitonin, hCTR (Fig. 2B) receptors. Fig. 2C provides the sequence of the amylin analogs

Figs. 3A-3C provides the results of an *in vitro* analysis of amylin analogs 5079 (SEQ ID NO: 31), 5092 (SEQ ID NO: 32) and 5093 (SEQ ID NO: 33), 5136 (SEQ ID NO: 40), 5137 (SEQ ID NO: 41), and 5138 (SEQ ID NO: 42) at the human amylin, hAMY3R (Fig. 3A) or human calcitonin, hCTR (Fig. 3B) receptors, Fig. 3C provides the sequence of the amylin analogs

Fig. 4 provides the results of an *in vivo* experiment where rats are administered a single subcutaneous dose (at 2 nmol/kg) of amylin analogs 5079 (SEQ ID NO: 31), 5092 (SEQ ID NO: 32) and 5093 (SEQ ID NO: 33). The percent change in body weight was measured over time.

Figs. 5A-5C provide the results of an *in vitro* assay measuring the activity of amylin analogs 5079 (SEQ ID NO: 31), 5113 (SEQ ID NO: 34), 5114 (SEQ ID NO: 35), and 5115 (SEQ ID NO: 36) at the human amylin, hAMY3R (Fig. 5A) or human calcitonin, hCTR (Fig. 5B) receptors. Fig. 5C provides the sequence of the amylin analogs.

Figs. 6A-6B provide the results of an *in vivo* experiment where rats are administered a single subcutaneous dose (at 2 nmol/kg) of amylin analogs 5071 (SEQ ID NO: 30), 5079 (SEQ ID NO: 31), 5113 (SEQ ID NO: 34), 5114 (SEQ ID NO: 35) and 5115 (SEQ ID NO: 36), with the data provided in Fig. 6A. The percent change in body weight was measured over time. Fig. 6B provides the sequence of the amylin analogs.

Figs. 7A-7C provide the results of an *in vitro* assay measuring the activity of amylin analogs 5071 (cagrilintide; (SEQ ID NO: 30)), 5113 (SEQ ID NO: 34), 5129 (SEQ ID NO: 38) and 5139 (SEQ ID NO: 43) at the human amylin, hAMY3R (Fig.

7A) or human calcitonin, hCTR (Fig. 7B) receptors. Fig. 7C provides the sequence of the amylin analogs and the EC₅₀ values for each analog at the respective receptors.

Figs. 8A-8C provide the results of an *in vivo* experiment where rats are administered a single subcutaneous dose (at 2 nmol/kg) of amylin analogs 5071 (SEQ ID NO: 30), 5129 (SEQ ID NO: 38) and 5139 (SEQ ID NO: 43). The percent change in body weight (Fig. 8A) and change in food intake (Fig. 8B) was measured over time. Fig. 8C provides the sequence of the amylin analogs.

Figs. 9A-9C provide the results of an *in vivo* experiment where rats are administered a single subcutaneous dose (at 2 & 4 nmol/kg) of amylin analogs 5079 (SEQ ID NO: 31), 5113 (SEQ ID NO: 34) and 5129 (SEQ ID NO: 38). The percent change in body weight (Fig. 9A) and change in food intake (Fig. 9B) was measured over time. Fig. 9C provides the sequence of the amylin analogs.

Fig. 10 provides the results of an *in vivo* experiment where diet-induced obese (DIO) rats are administered a single subcutaneous dose (at 0.5, 1 and 2 nmol/kg) of amylin analog 5129 (SEQ ID NO: 38). Body weight over time was measured.

Fig. 11A and 11B an *in vivo* experiment where diet-induced obese (DIO) rats are administered a single subcutaneous dose (at 0.5, 1 and 2 nmol/kg) of amylin analogs 5129 (SEQ ID NO: 38). The percent change in body weight (Fig. 11A) and food intake (Fig. 11B) over time was measured.

Fig. 12 provides the results of an Inotiv NHP (cynomolgus monkey) PK Analysis Study for amylin analogs 5071 (SEQ ID NO: 30), 5129 (SEQ ID NO: 38), 5139 (SEQ ID NO: 43) and 5144 (SEQ ID NO: 47). The structure for 5144 is O-XCNTATCATQRLAEFLRHSSNFGPILZZTNVGSNTZ-NH₂. (SEQ ID NO: 47). The peptides were subcutaneously injected at 40nmol/kg and the plasma concentration over the course of two weeks was assessed by LCMS. Z = hydroxyproline.

Figs. 13A-13C provide the results of an *in vitro* assay measuring the activity of amylin analogs 5071 (cagrilintide (SEQ ID NO: 30)), 5129 (SEQ ID NO: 38), 5139 (SEQ ID NO: 43) and 5140 (SEQ ID NO: 44) at the human amylin, hAMY3R (Fig. 13A) or human calcitonin, hCTR (Fig. 13B) receptors. Fig. 13C provides the sequence of the amylin analogs and the EC₅₀ values for each analog at the respective receptors.

Figs. 14A-14C provide the results of an *in vitro* assay measuring the activity of amylin analogs 5113 (SEQ ID NO: 34), 5128 (SEQ ID NO: 37), 5129 (SEQ ID

NO: 38) and 5130 (SEQ ID NO: 39) at the human amylin, hAMY3R (Fig. 14A) or human calcitonin, hCTR (Fig. 14B) receptors. Fig. 14C provides the sequence of the amylin analogs.

Fig. 15 presents the data from an *in vivo* experiment where diet-induced obese (DIO) rats are administered a single subcutaneous dose of amylin analogs 5113 (SEQ ID NO: 34), 5128 (SEQ ID NO: 37), 5129 (SEQ ID NO: 38) and 5130 (SEQ ID NO: 39) at 2nmol/kg. The percent change in body weight over time was measured.

Figs. 16A-16D provide the results of an *in vitro* assay measuring the activity of amylin analogs 5129 (SEQ ID NO: 38), 5157 (SEQ ID NO: 57), 5158 (SEQ ID NO: 58) and 5159 (SEQ ID NO: 59) at the human amylin, hAMY3R (Fig. 16A) or human calcitonin, hCTR (Fig. 16B) receptors. Fig. 16C provides the EC₅₀ values for each analog at the respective receptors and Fig. 16D provides the sequence of the amylin analogs.

Figs. 17A-17C provides the structure and sequence of the DACRA isoacyl analogs (Fig. 17A) and the results of an *in vitro* assay measuring the activity of amylin analogs 5129 (SEQ ID NO: 38), 5143 (SEQ ID NO: 46) and 5145 (SEQ ID NO: 48) at the human amylin, hAMY3R (Fig. 17B) or human calcitonin, hCTR (Fig. 17C) receptors. 5143A and 5143B designated in the figures represent two separate runs with compound 5143.

Figs. 18A-18C provide the results of an *in vitro* assay measuring the activity of amylin analogs 5071 (SEQ ID NO: 30), 5129 (SEQ ID NO: 38), 5139 (SEQ ID NO: 43), 5142 (SEQ ID NO: 45), 5143 (SEQ ID NO: 46) and 5144 (SEQ ID NO: 47) at the human amylin, hAMY3R (Fig. 18A) or human calcitonin, hCTR (Fig. 18B) receptors. Fig. 18C provides the sequence of the amylin analogs and the EC₅₀ values for each analog at the respective receptors.

Figs. 19A-19C provide the results of an *in vitro* assay measuring the activity of amylin analogs 5071 (SEQ ID NO: 30), 5129 (SEQ ID NO: 38), 5143 (SEQ ID NO: 46), 5145 (SEQ ID NO: 48), 5146 (SEQ ID NO: 49) and 5147 (SEQ ID NO: 50) at the human amylin, hAMY3R (Fig. 19A) or human calcitonin, hCTR (Fig. 19B) receptors. The sequence of the amylin analogs 5071 (SEQ ID NO: 30), 5129 (SEQ ID NO: 38), 5143 (SEQ ID NO: 46), 5145 (SEQ ID NO: 48), 5146 (SEQ ID NO: 49) and 5147 (SEQ ID NO: 50) is provided (Fig. 19C).

Fig. 20 presents the data from an *in vivo* experiment where diet-induced obese (DIO) rats are administered a single subcutaneous dose of amylin analogs 5071 (SEQ

ID NO: 30), 5129 (SEQ ID NO: 38), 5143 (SEQ ID NO: 46), 5145 (SEQ ID NO: 48) and 5146 (SEQ ID NO: 49) at 2nmol/kg. The percent change in body weight over time was measured.

Figs. 21A-21C provide the results of an *in vitro* assay measuring the activity of amylin analogs 5143 (SEQ ID NO: 46), 5145 (SEQ ID NO: 48), 5148 (SEQ ID NO: 51), 5149 (SEQ ID NO: 52), 5150 (SEQ ID NO: 53) and 5151 (SEQ ID NO: 54) at the human amylin, hAMY3R (Fig. 21A) or human calcitonin, hCTR (Fig. 21B) receptors. Fig. 21C provides the sequence of the amylin analogs.

Fig. 22 provides the results of an *in vivo* experiment where diet-induced obese (DIO) rats are administered a single subcutaneous dose (2 and 20nmol/kg) of amylin analogs 5143 (SEQ ID NO: 46), 5148 (SEQ ID NO: 51), 5150 (SEQ ID NO: 53), 5152 (SEQ ID NO: 55) and 5160 (SEQ ID NO: 60). Body weight over time was measured.

Figs. 23A-23D provide the results of an *in vitro* assay measuring the activity of amylin analogs 5129 (SEQ ID NO: 38), 5155 (SEQ ID NO: 56), 5161 (SEQ ID NO: 61), 5163 (SEQ ID NO: 62), 5168 (SEQ ID NO: 69) and 5169 (SEQ ID NO: 70) at the human amylin, hAMY3R (Fig. 23A) or human calcitonin, hCTR (Fig. 23B) receptors. Fig. 23C provides the sequence of the amylin analogs and the EC₅₀ values for each analog at the respective receptors is provided in Fig. 23D.

Figs. 24A-24D provide the results of an *in vitro* assay measuring the activity of amylin analogs 5129 (SEQ ID NO: 38), 5155 (SEQ ID NO: 56), 5171 (SEQ ID NO: 71), 5173 (SEQ ID NO: 72), 5177 (SEQ ID NO: 76) and 5178 (SEQ ID NO: 77) at the human amylin, hAMY3R (Fig. 24A) or human calcitonin, hCTR (Fig. 24B) receptors. Fig. 24C provides the sequence of the amylin analogs and the EC₅₀ values for each analog at the respective receptors is provided in Fig. 24D.

Figs. 25A-25C provide the results of an *in vitro* assay measuring the activity of amylin analogs 5129 (SEQ ID NO: 38), 5160 (SEQ ID NO: 60), 5164 (SEQ ID NO: 63), 5165 (SEQ ID NO: 64), 5166 (SEQ ID NOs: 65 and 66) and 5167 (SEQ ID NOs: 67 and 68) at the human amylin, hAMY3R (Fig. 25A) or human calcitonin, hCTR (Fig. 25B) receptors. Fig. 25C provides the sequence of the amylin analogs.

Fig. 26 presents the data from an *in vivo* experiment where diet-induced obese (DIO) rats are administered a single subcutaneous dose of amylin analogs 5129 (SEQ ID NO: 38), 5143 (SEQ ID NO: 46), 5164 (SEQ ID NO: 63), 5165 (SEQ ID NO: 64)

and 5167 (SEQ ID NOs: 67 and 68) at 2nmol/kg. The percent change in body weight over time was measured.

Figs. 27A-27D provide the results of an *in vitro* assay measuring the activity of amylin analogs 5163 (SEQ ID NO: 62), 5168 (SEQ ID NO: 69), 5179 (SEQ ID NO: 78), 5187 (SEQ ID NOs: 82 and 83), 5188 (SEQ ID NOs: 84 and 85) and 5190 (SEQ ID NOs: 89 and 90) at the human amylin, hAMY3R (Fig. 27A) or human calcitonin, hCTR (Fig. 27B) receptors. Fig. 27C provides the sequence of the amylin analogs, noting the internal deletion of amino acids in analogs relative to Cagrilinitide (analog number 5071, SEQ ID NO: 30). The EC₅₀ values for each analog at the respective receptors is provided in Fig. 27D.

Fig. 28 presents the data from an *in vivo* experiment where diet-induced obese (DIO) rats are administered a single subcutaneous dose of amylin analogs 5129 (SEQ ID NO: 38), 5188 (SEQ ID NOs: 84 and 85), and 5190 (SEQ ID NOs: 89 and 90) at 2nmol/kg. The percent change in body weight over time was measured.

Figs. 29A-29D provide the results of an *in vitro* assay measuring the activity of amylin analogs 5196 (SEQ ID NO: 100), 5190 (SEQ ID NOs: 89 and 90), 5129 (SEQ ID NO: 38), 5113 (SEQ ID NO: 34), 5079 (SEQ ID NO: 31) and 5171 (SEQ ID NO: 71) at the human amylin, hAMY3R (Fig. 29A) or human calcitonin, hCTR (Fig. 29B) receptors. Fig. 29C provides the sequence of the amylin analogs, noting the internal deletion of amino acids in analogs relative to Cagrilinitide (analog number 5071 (SEQ ID NO: 30)). The EC₅₀ values for each analog at the respective receptors is provided in Fig. 29D.

Fig. 30 presents the data from an *in vivo* experiment where diet-induced obese (DIO) rats are administered a single subcutaneous dose of amylin analogs 5196 (SEQ ID NO: 100), 5190 (SEQ ID NOs: 89 and 90), 5129 (SEQ ID NO: 38), 5113 (SEQ ID NO: 34), 5079 (SEQ ID NO: 31) and 5171 (SEQ ID NO: 71) at 2nmol/kg. The percent change in body weight over time was measured.

Fig. 31 provides the results of an Inotiv NHP (cynomolgus monkey) PK Analysis Study for amylin analogs 5164 (SEQ ID NO: 63), 5165 (SEQ ID NO: 64), 5166 (SEQ ID NOs: 65 and 66), 5167 (SEQ ID NOs: 67 and 68) and 5190 (SEQ ID NOs: 89 and 90). The peptides were subcutaneously injected at 40nmol/kg and the plasma concentration over the course of two weeks was assessed by LCMS. Z = hydroxyproline.

Fig. 32 is a schematic drawing showing how introduction of a mid-sequence branch and the use of mini-peg spacers can be utilized to create conjugates with other bioactive compounds such as incretins. Fig. 32 provides the structure of a conjugate formed between a DACRA peptide of the present invention (MBX 5167; SEQ ID NOs: 67 and 68) and an incretin (Semaglutide; SEQ ID NO: 142) wherein the carboxy terminus of the incretin is covalently linked to the alpha amine of an Orn residue at position 28 of the DACRA peptide via a PEG spacer and the side chain delta amine of the Orn residue is linked to the amino acid at position 27 of the DACRA peptide via a PEG spacer. The structure shown in Fig. 32 can be represented by the following designation: H-Aib-EGTFTSDVSSYLEGQAAKEFIAWLVRGRG-Peg8-Orn((βK-CNTATCATQRLAEFLRHSSNNFGAIL-Peg8)STNVGSNT-Hyp-amide.

Figs. 33A-33D provide the results of an *in vitro* assay measuring the activity of amylin analogs 5155 (SEQ ID NO: 56), 5167 (SEQ ID NOs: 67 and 68), 5171 (SEQ ID NO: 71), and 5175 (SEQ ID NOs: 73-75) at the human amylin, hAMY3R (Fig. 33A) or human calcitonin, hCTR (Fig. 33B) receptors. Fig. 33C provides the sequence of the amylin analogs and the EC₅₀ values for each analog at the respective receptors is provided in Fig 33D.

Figs. 34A & 34B provide the results of an *in vitro* assay measuring the activity of semaglutide (MBX 1420; SEQ ID NO: 19), amylin analogs 5167 (SEQ ID NOs: 67 and 68) and 5175 (SEQ ID NOs: 73-75) at the human GLP-1R (Fig. 34A). The sequence of the amylin analogs and the EC₅₀ values at the GLP-1 receptor are provided in Fig. 34B.

Figs. 35A-35D provide the results of an *in vitro* assay measuring the activity of amylin analogs 1420 (SEQ ID NO: 19), 5129 (SEQ ID NO: 38), 5167 (SEQ ID NOs: 67 and 68), 5175, (SEQ ID NOs: 73-75) 5186 (SEQ ID NOs: 79-81) and 5189 (SEQ ID NOs: 86-88) at the human amylin, hAMY3R (Fig. 35A) or human calcitonin, hCTR (Fig. 35B) receptors. Fig. 35C provides the sequence of the amylin analogs and Fig. 35D provides the EC₅₀ values for each analog at the respective receptors.

Figs. 36A-36D provide the results of an *in vitro* assay measuring the activity of amylin analogs 1420 (SEQ ID NO: 19), s (SEQ ID NO: 67 and 68), 5175 (SEQ ID NOs: 73-75), 5186 (SEQ ID NOs: 79-81), 5189 (SEQ ID NOs: 86-88) and 5129 (SEQ ID NO: 38) at the human amylin, hAMY3R (Fig. 36A) or human calcitonin, hCTR

(Fig. 36B) receptors. Fig. 36C provides the sequence of the amylin analogs and the EC₅₀ values for each analog at the respective receptors are shown in Fig. 36D.

Fig. 37 provide the results of an *in vitro* assay measuring the activity of semaglutide (MBX 1420 (SEQ ID NO: 19)), and amylin analogs 5167 (SEQ ID NOs: 67 and 68), 5175 (SEQ ID NOs: 73-75), 5186 (SEQ ID NOs: 79-81), 5189 (SEQ ID NOs: 86-88) and 5129 (SEQ ID NO: 38) at the human GLP-1R. The sequence of the amylin analogs and the EC₅₀ values at the GLP-1 receptor are provided.

Figs. 38A-38D provide the results of an *in vitro* assay measuring the activity of amylin analogs 5167 (SEQ ID NOs: 67 and 68), 5129 (SEQ ID NO: 38), 4244 (SEQ ID NO: 21) and 5191 (SEQ ID NOs: 91-93) at the human amylin, hAMY3R (Fig. 38A) or human calcitonin, hCTR (Fig. 38B) receptors. Fig. 38C provides the sequence of the amylin analogs, and the EC₅₀ values for each analog at the respective receptors is provided in Fig 38D.

Figs. 39A-39E provide the results of an *in vitro* assay measuring the activity of 4244 (Retatrutide; SEQ ID NO: 21) and amylin analogs 5167 (SEQ ID NOs: 67 and 68) and 5191 (SEQ ID NOs: 91-93), at the human glucagon receptor (Fig 39A), the hGIP receptor (Fig. 39B) and the hGLP-1 receptor (Fig. 39C). The sequence of the amylin analogs is provided in Fig. 39D, and the EC₅₀ values at the GLP-1 receptor are provided in Fig. 39E.

Figs. 40A-40D provide the results of an *in vitro* assay measuring the activity of amylin analogs 5129 (SEQ ID NO: 38), 5186 (SEQ ID NOs: 79-81), 5190, (SEQ ID NOs: 89 and 90) 5193 (SEQ ID NOs: 94-96) and 5194 (SEQ ID NOs: 97-99), relative to analog 1420 (SEQ ID NO: 19), at the human amylin, hAMY3R (Fig. 40A) or human calcitonin, hCTR (Fig. 40B) receptors. Fig. 40C provides the sequence of the amylin analogs, and the EC₅₀ values for each analog at the respective receptors is provided in Fig. 40D.

Fig. 41A-41D provide the results of an *in vitro* assay measuring the activity of amylin analogs 5186 (SEQ ID NOs: 79-81), 5190 (SEQ ID NOs: 89 and 90), 5193 (SEQ ID NOs: 94-96) and 5194 (SEQ ID NOs: 97-99) at the human GLP-1 receptor (Fig 41A) and the mouse GLP-1 receptor (Fig 41B). The sequence of the amylin analogs are provided in Fig 41C and the EC₅₀ values at the GLP-1 receptor in Fig 41D.

Figs. 42A-42D provide the results of an *in vitro* assay measuring the activity of amylin analogs 5129 (SEQ ID NO: 38), 5190 (SEQ ID NOs: 89 and 90), 5194

(SEQ ID NOs: 97-99), 5197 (SEQ ID NOs: 101-103), 5198 (SEQ ID NOs: 104-106) and 1420 (SEQ ID NO: 19) at the human amylin, hAMY3R (Fig. 42A) or human calcitonin, hCTR (Fig. 42B) receptors. Fig. 42C provides the sequence of the amylin analogs, and the EC₅₀ values for each analog at the respective receptors is provided in Fig. 42D.

Fig. 43 provides the results of an *in vitro* assay measuring the activity of amylin analogs 5194 (SEQ ID NOs: 97-99), 5197 (SEQ ID NOs: 101-103) and 1420 (SEQ ID NO: 19) at the human GLP-1 receptor.

Fig. 44 provides the results of an *in vitro* assay measuring the activity of amylin analogs 5194 (SEQ ID NOs: 97-99), 5198 (SEQ ID NOs: 104-106) and 1420 (SEQ ID NO: 19) at the human GLP-1 receptor.

Figs. 45A-45D provide the results of an *in vitro* assay measuring the activity of amylin analogs 5129 (SEQ ID NO: 38), 5190 (SEQ ID NOs: 89 and 90), 5194 (SEQ ID NOs: 97-99), 5199 (SEQ ID NOs: 107-109) and 1420 (SEQ ID NO: 19) at the human amylin, hAMY3R (Fig. 45A) or human calcitonin, hCTR (Fig. 45B) receptors. Fig. 45C provides the sequence of the amylin analogs, and the EC₅₀ values for each analog at the respective receptors is provided in Fig. 45D.

Fig. 46A-46D provide the results of an *in vitro* assay measuring the activity of tirzepatide (MBX 4060 (SEQ ID NO: 20)), and amylin analogs 5190 (SEQ ID NOs: 89 and 90) and 5199 (SEQ ID NOs: 107-109) at the human GLP-1 receptor (Fig. 46A) and at the human GIP receptor (Fig. 46B). The sequence of the amylin analogs is provided in Fig. 46C, and the EC₅₀ values at the GLP-1 receptor are provided in Fig. 46D.

Figs. 47A-47D provide the results of an *in vitro* assay measuring the activity of amylin analogs 5190 (SEQ ID NOs: 89 and 90), 5191 (SEQ ID NOs: 91-93), 5200 (SEQ ID NOs: 110-112), and 4244 (Retatrutide; (SEQ ID NO: 21)) at the human amylin, hAMY3R (Fig. 47A) or human calcitonin, hCTR (Fig. 47B) receptors. Fig. 47C provides the sequence of the amylin analogs, and the EC₅₀ values for each analog at the respective receptors is provided in Fig. 47D.

Figs. 48A-48D provide the results of an *in vitro* assay measuring the activity of amylin analogs 5190 (SEQ ID NOs: 89 and 90), 5204 (SEQ ID NOs: 113 and 114), 5212 (SEQ ID NOs: 115-117), 5213 (SEQ ID NOs: 118-120), 5215 (SEQ ID NOs: 124-126) and 1420 (SEQ ID NO: 19) at the human amylin, hAMY3R (Fig. 48A) or human calcitonin, hCTR (Fig. 48B) receptors. Fig. 48C provides the sequence of the

amylin analogs, and the EC₅₀ values for each analog at the respective receptors is provided in Fig. 48D.

Fig.49A-49E provide the results of an *in vitro* assay measuring the activity of amylin analogs 5190 (SEQ ID NOs: 89 and 90), 5204 (SEQ ID NOs: 113 and 114),
5 5212 (SEQ ID NOs: 115-117), 5213 (SEQ ID NOs: 118-120), 5215 (SEQ ID NOs: 124-126) and 4301 (SEQ ID NO: 24) at the human glucagon receptor (Fig. 49A), the hGIP receptor (Fig. 49B) and the hGLP-1 receptor (Fig. 49C). The sequence of the amylin analogs is provided in Fig. 49D and the EC₅₀ values at the GLP-1 receptor are provided in Fig. 49E.

10 Figs. 50A-50D provide the results of an *in vitro* assay measuring the activity of amylin analogs 5190 (SEQ ID NOs: 89 and 90), 5215 (SEQ ID NOs: 124-126), 5233 (SEQ ID NOs: 127-129), 5234 (SEQ ID NOs: 130-132), 5235 (SEQ ID NOs: 133-135) and 4301 (SEQ ID NO: 24) at the human amylin, hAMY3R (Fig. 50A) or human calcitonin, hCTR (Fig. 50B) receptors. Fig. 50C provides the sequence of the
15 amylin analogs, and the EC₅₀ values for each analog at the respective receptors is provided in Fig. 50D.

Fig.51A-51E provide the results of an *in vitro* assay measuring the activity of amylin analogs 4301 (SEQ ID NO: 24), 5215 (SEQ ID NOs: 124-126), 5233 (SEQ ID NOs: 127-129), 5234 (SEQ ID NOs: 130-132), and 5235 (SEQ ID NOs: 133-135) at
20 the human glucagon receptor (Fig. 51A), the hGIP receptor (Fig. 51B) and the hGLP-1 receptor (Fig. 51C). The sequence of the amylin analogs is provided in Fig. 51D and the EC₅₀ values at the GLP-1 receptor are provided in Fig. 51E.

Figs. 52A-52D provide the results of an *in vitro* assay measuring the activity of amylin analogs 5215 (SEQ ID NOs: 124-126), 5239 (SEQ ID NOs: 136-138), 5240
25 (SEQ ID NOs: 139-141), 5190 (SEQ ID NOs: 89 and 90) and 4301 (SEQ ID NO: 24) at the human amylin, hAMY3R (Fig. 52A) or human calcitonin, hCTR (Fig. 52B) receptors. Fig. 52C provides the sequence of the amylin analogs, and the EC₅₀ values for each analog at the respective receptors are provided in Fig. 52D.

Fig.53A-53E provide the results of an *in vitro* assay measuring the activity of
30 amylin analogs 5215 (SEQ ID NOs: 124-126), 5239 (SEQ ID NOs: 136-138), 5240 (SEQ ID NOs: 139-141), 5190 (SEQ ID NOs: 89 and 90) and 4301 (SEQ ID NO: 24) at the human glucagon receptor (Fig. 53A), the hGIP receptor (Fig. 53B) and the hGLP-1 receptor (Fig. 53C). The sequence of the amylin analogs is provided in Fig. 53D and the EC₅₀ values at the GLP-1 receptor are provided in Fig. 53E.

Fig. 54 provides the results of an Inotiv NHP (cynomolgus monkey) PK Analysis Study for amylin analogs 5190 (SEQ ID NOs: 89 and 90), 5194 (SEQ ID NOs: 97-99), 5198 (SEQ ID NOs: 104-106), 5199 (SEQ ID NOs: 107-109) and 5200 (SEQ ID NOs: 110-112). The peptides were subcutaneously injected at 40nmol/kg and the plasma concentration over the course of one week was assessed by LCMS.

Fig. 55 provides the results of an Inotiv NHP (cynomolgus monkey) PK Analysis Study for amylin analogs 5190 (SEQ ID NOs: 89 and 90), 5194 (SEQ ID NOs: 97-99), 5198 (SEQ ID NOs: 104-106), 5199 (SEQ ID NOs: 107-109) and 5200 (SEQ ID NOs: 110-112). The peptides were subcutaneously injected at 40nmol/kg and the plasma concentration over the course of one week was assessed by LCMS.

Fig. 56 provides the results of an Inotiv NHP (cynomolgus monkey) PK Analysis Study for amylin analogs 4301 (SEQ ID NO: 24), 5212 (SEQ ID NOs: 115-117), 5213 (SEQ ID NOs: 118-120) and 5215 (SEQ ID NOs: 124-126). The peptides were subcutaneously injected at 30nmol/kg and the plasma concentration over the course of one week was assessed by LCMS.

Fig. 57 provides the results of an Inotiv NHP (cynomolgus monkey) PK Analysis Study for amylin analogs 4301 (SEQ ID NO: 24), 5212 (SEQ ID NOs: 115-117), 5213 (SEQ ID NOs: 118-120) and 5215 (SEQ ID NOs: 124-126). The peptides were subcutaneously injected at 30nmol/kg and the plasma concentration over the course of one week was assessed by LCMS.

Fig. 58 presents the data from an *in vivo* experiment where diet-induced obese (DIO) rats are administered a single subcutaneous dose of amylin analogs 5129 (SEQ ID NO: 38) at 2nmol/kg, or 4301 (SEQ ID NO: 24), 5212 (SEQ ID NOs: 115-117), 5213 (SEQ ID NOs: 118-120), 5214 (SEQ ID NOs: 121-123) and 5215 (SEQ ID NOs: 124-126) at 5nmol/kg. The percent change in body weight over time was measured.

Fig. 59 presents the data from an *in vivo* experiment where diet-induced obese (DIO) rats are administered a single subcutaneous dose of amylin analogs 5129 (SEQ ID NO: 38) at 10nmol/kg, or 4301 (SEQ ID NO: 24), 5212 (SEQ ID NOs: 115-117), 5213 (SEQ ID NOs: 118-120), 5214 (SEQ ID NOs: 121-123) and 5215 (SEQ ID NOs: 124-126) at 5nmol/kg. The percent change in body weight over time was measured.

Fig. 60 presents the data from an *in vivo* experiment where diet-induced obese (DIO) rats are administered a single subcutaneous dose of amylin analogs 5190 (SEQ

ID NOs: 89 and 90) at 2nmol/kg, or 5215 (SEQ ID NOs: 124-126), 5233 (SEQ ID NOs: 127-129), 5234 (SEQ ID NOs: 130-132) and 5235 (SEQ ID NOs: 133-135) at 5nmol/kg. The percent change in body weight over time was measured.

Fig. 61 presents the data from an *in vivo* experiment where diet-induced obese (DIO) rats are administered a single subcutaneous dose of amylin analogs 4301 (SEQ ID NO: 24), 5190 (SEQ ID NOs: 89 and 90), 5215 (SEQ ID NOs: 124-126), 5239 (SEQ ID NOs: 136-138) and 5240 (SEQ ID NOs: 139-141) at 2nmol/kg and a combination of 5190 + 4301 at 2nmol/kg. The percent change in body weight over time was measured.

Fig. 62 presents the data from an *in vivo* experiment where diet-induced obese (DIO) rats are administered a single subcutaneous dose of amylin analogs 4301 (SEQ ID NO: 24), 5190 (SEQ ID NOs: 89 and 90), 5215 (SEQ ID NOs: 124-126), 5239 (SEQ ID NOs: 136-138) and 5240 (SEQ ID NOs: 139-141) at 2nmol/kg and a combination of 5190 + 4301 at 2nmol/kg. The percent change in body weight over time was measured.

Fig. 63 provides a table of DACRA peptides and conjugate derivatives and prodrugs thereof as disclosed herein along with their compound reference number and corresponding sequence identifiers.

DETAILED DESCRIPTION

DEFINITIONS

In describing and claiming the invention, the following terminology will be used in accordance with the definitions set forth below.

The term "about" as used herein means greater or lesser than the value or range of values stated by 10 percent but is not intended to designate any value or range of values to only this broader definition. Each value or range of values preceded by the term "about" is also intended to encompass the embodiment of the stated absolute value or range of values.

As used herein, the term "pharmaceutically acceptable carrier" includes any of the standard pharmaceutical carriers, such as a phosphate buffered saline solution, water, emulsions such as an oil/water or water/oil emulsion, and various types of wetting agents. The term also encompasses any of the agents approved by a regulatory agency of the US Federal government or listed in the US Pharmacopeia for use in animals, including humans.

As used herein the term "pharmaceutically acceptable salt" refers to salts of compounds that retain the biological activity of the parent compound, and which are not biologically or otherwise undesirable. Many of the compounds disclosed herein are capable of forming acid and/or base salts by virtue of the presence of amino
5 and/or carboxyl groups or groups similar thereto.

Pharmaceutically acceptable base addition salts can be prepared from inorganic and organic bases. Salts derived from inorganic bases, include by way of example only, sodium, potassium, lithium, ammonium, calcium and magnesium salts. Salts derived from organic bases include, but are not limited to, salts of primary,
10 secondary and tertiary amines.

Pharmaceutically acceptable acid addition salts may be prepared from inorganic and organic acids. Salts derived from inorganic acids include hydrochloric acid, hydrobromic acid, sulfuric acid, nitric acid, phosphoric acid, and the like. Salts derived from organic acids include acetic acid, propionic acid, glycolic acid, pyruvic
15 acid, oxalic acid, malic acid, malonic acid, succinic acid, maleic acid, fumaric acid, tartaric acid, citric acid, benzoic acid, cinnamic acid, mandelic acid, methanesulfonic acid, ethanesulfonic acid, p-toluene-sulfonic acid, salicylic acid, and the like.

As used herein, the term "treating" includes alleviation of the symptoms associated with a specific disorder or condition, or the eliminating said symptoms.
20 For example, as used herein the term "treating diabetes" will refer in general to altering glucose blood levels in the direction of normal levels and may include increasing or decreasing blood glucose levels depending on a given situation.

As used herein an "effective" amount or a "therapeutically effective amount" of a glucagon peptide refers to a nontoxic but sufficient amount of the peptide to
25 provide the desired effect. For example, one desired effect would be the prevention or treatment of hypoglycemia, as measured, for example, by an increase in blood glucose level. An alternative desired effect for the glucagon peptides of the present disclosure would include treating hyperglycemia, e.g., as measured by a change in blood glucose level closer to normal, or inducing weight loss/preventing weight gain, e.g., as
30 measured by reduction in body weight, or preventing or reducing an increase in body weight, or normalizing body fat distribution. The amount that is "effective" will vary from subject to subject, depending on the age and general condition of the individual, mode of administration, and the like. Thus, it is not always possible to specify an exact "effective amount." However, an appropriate "effective" amount in any

individual case may be determined by one of ordinary skill in the art using routine experimentation.

The term, "parenteral" means not through the alimentary canal but by some other route such as subcutaneous, intramuscular, intraspinal, or intravenous.

5 As used herein, the term "purified" and like terms relate to the isolation of a molecule or compound in a form that is substantially free of contaminants normally associated with the molecule or compound in a native or natural environment.

As used herein, the term "purified" does not require absolute purity; rather, it is intended as a relative definition. The term "purified polypeptide" is used herein to
10 describe a polypeptide which has been separated from other compounds including, but not limited to nucleic acid molecules, lipids and carbohydrates.

The term "isolated" requires that the referenced material be removed from its original environment (e.g., the natural environment if it is naturally occurring). For example, a naturally occurring polynucleotide present in a living animal is not
15 isolated, but the same polynucleotide, separated from some or all of the coexisting materials in the natural system, is isolated.

As used herein, the term "peptide" encompasses a sequence of 2 or more amino acids and typically less than 50 amino acids, wherein the amino acids are naturally occurring or non-naturally occurring amino acids. Non-naturally occurring
20 amino acids refer to amino acids that do not naturally occur in vivo but which, nevertheless, can be incorporated into the peptide structures described herein.

As used herein, the terms "polypeptide" and "protein" are terms that are used interchangeably to refer to a polymer of amino acids, without regard to the length of the polymer. Typically, polypeptides and proteins have a polymer length that is
25 greater than that of "peptides."

As used herein an amino acid "modification" refers to a substitution, addition or deletion of an amino acid, and includes substitution with or addition of any of the 20 amino acids commonly found in human proteins, as well as atypical or non-naturally occurring amino acids. Commercial sources of atypical amino acids include
30 Sigma-Aldrich (Milwaukee, WI), ChemPep Inc. (Miami, FL), and Genzyme Pharmaceuticals (Cambridge, MA). Atypical amino acids may be purchased from commercial suppliers, synthesized de novo, or chemically modified or derivatized from other amino acids.

As used herein an amino acid "substitution" refers to the replacement of one amino acid residue by a different amino acid residue.

As used herein, the term "conservative amino acid substitution" is defined herein as exchanges within one of the following five groups:

- 5 I. Small aliphatic, nonpolar or slightly polar residues:
Ala, Ser, Thr, Pro, Gly and hydroxyproline;
- II. Polar, negatively charged residues and their amides and esters:
Asp, Asn, Glu, Gln, cysteic acid and homocysteic acid;
- III. Polar, positively charged residues:
10 His, Arg, Lys; Ornithine (Orn)
- IV. Large, aliphatic, nonpolar residues:
Met, Leu, Ile, Val, Cys, Norleucine (Nle), homocysteine
- V. Large, aromatic residues:
Phe, Tyr, Trp, acetyl phenylalanine

15

As used herein a general reference to a peptide is intended to encompass peptides that have modified amino and carboxy termini. For example, an amino acid chain comprising an amide group in place of the terminal carboxylic acid is intended to be encompassed by an amino acid sequence designating the standard amino acids.

20

As used herein a Dual Amylin Calcitonin Receptor Agonists (DACRA) is a peptide analog of amylin that has agonist activity at both the calcitonin receptor and the amylin receptor and has at least 10% the agonist activity of native amylin and native calcitonin at their respective receptors.

25 As used herein an incretin peptide encompasses peptides having biological activity as agonists at any one or more of the glucagon, GLP-1, GLP-2, and GIP receptors.

As used herein a "linker" is a bond, molecule or group of molecules that binds two separate entities to one another. Linkers may provide for optimal spacing of the two entities or may further supply a labile linkage that allows the two entities to be
30 separated from each other. Labile linkages include photocleavable groups, acid-labile moieties, base-labile moieties and enzyme-cleavable groups.

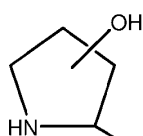
As used herein a "dimer" is a complex comprising two subunits covalently bound to one another via a linker. The term dimer, when used absent any qualifying language, encompasses both homodimers and heterodimers. A homodimer comprises

two identical subunits, whereas a heterodimer comprises two subunits that differ, although the two subunits are substantially similar to one another.

As used herein the term "charged amino acid" refers to an amino acid that comprises a side chain that is negatively charged (i.e., de-protonated) or positively charged (i.e., protonated) in aqueous solution at physiological pH. For example, negatively charged amino acids include aspartic acid, glutamic acid, cysteic acid, homocysteic acid, and homoglutamic acid, whereas positively charged amino acids include arginine, lysine and histidine. Charged amino acids include the charged amino acids among the 20 amino acids commonly found in human proteins, as well as atypical or non-naturally occurring amino acids.

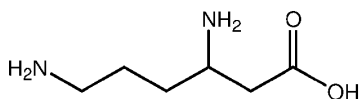
As used herein the term "acidic amino acid" refers to an amino acid that comprises a second acidic moiety, including for example, a carboxylic acid or sulfonic acid group.

As used herein the term "hydroxyproline" absent any further elaboration encompasses compounds having the structure:

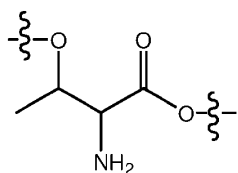


and includes the specific compounds 3- hydroxyproline, 4- hydroxyproline and 5- hydroxyproline and all the allo-stereoisomers.

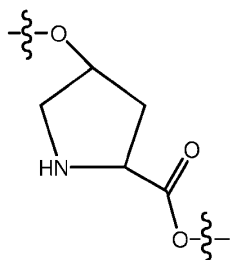
As used herein "beta-Lys" is an amino acid having the structure:



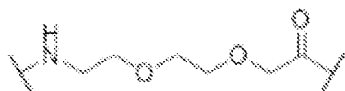
As used herein the term "isoacyl-peptide" defines a peptide having an ester linked amino acid. An "isoacyl-Thr" is a threonine residue that is linked to another amino acid via an ester bond, rather than a peptide bond, wherein the ester bond is formed using the side chain hydroxyl group of threonine. Accordingly, isoacyl-Thr has the structure:



, and an isoacyl-4-hydroxyproline comprises the structure:

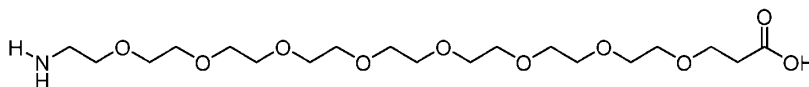


As used herein the term "miniPEG" or "OEG" defines a functionalized polyethylene compound comprising the structure:



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As used herein the term "PEG8" defines a functionalized polyethylene compound comprising the structure:



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As used herein the general term "polyethylene glycol chain" or "PEG chain", refers to mixtures of condensation polymers of ethylene oxide and water, in a branched or straight chain, represented by the general formula $H(OCH_2CH_2)_nOH$, wherein n is at least 9. Absent any further characterization, the term is intended to include polymers of ethylene glycol with an average total molecular weight selected from the range of 500 to 40,000 Daltons. "polyethylene glycol chain" or "PEG chain" is used in combination with a numeric suffix to indicate the approximate average molecular weight thereof. For example, PEG-5,000 (or PEG 5K) refers to polyethylene glycol chain having a total molecular weight average of about 5,000.

As used herein the term "pegylated" and like terms refers to a compound that has been modified from its native state by linking a polyethylene glycol chain to the

compound. A "pegylated DACRA peptide" is a glucagon peptide that has a PEG chain covalently bound to the DACRA peptide.

As used herein the term "phosphonic fatty acid" defines a lipid comprising both a carboxy acid and a phosphoric acid group wherein the carboxy acid and a
5 phosphoric acid groups are separated by a long carbon chain.

As used herein, the term "prodrug" is defined as any compound that undergoes chemical modification before exhibiting its full pharmacological effects.

As used herein the term "chemical cleavage" absent any further designation encompasses a non-enzymatic reaction that results in the breakage of a covalent
10 chemical bond.

Physiological conditions as disclosed herein are intended to include a temperature of about 35 to 40 °C and a pH of about 7.0 to about 7.4, and more typically include a pH of 7.2 to 7.4 and a temperature of 36 to 38 °C. Since physiological pH and temperature are tightly regulated in humans within a highly
15 defined range, the speed of conversion from dipeptide/drug complex (prodrug) to drug will exhibit high intra and interpatient reproducibility.

As used herein an "acylated" amino acid is an amino acid comprising an acyl group which is non-native to a naturally occurring amino acid, regardless by the means by which it is produced. Exemplary methods of producing acylated amino
20 acids and acylated peptides are known in the art and include acylating an amino acid before inclusion in the peptide or peptide synthesis followed by chemical acylation of the peptide. In some embodiments, the acyl group causes the peptide to have one or more of (i) a prolonged half-life in circulation, (ii) a delayed onset of action, (iii) an extended duration of action, and (iv) an improved resistance to proteases, such as
25 DPP-IV.

As used herein, an "alkylated" amino acid is an amino acid comprising an alkyl group which is non-native to a naturally occurring amino acid, regardless of the means by which it is produced. Exemplary methods of producing alkylated amino
30 acids and alkylated peptides are known in the art and include alkylating an amino acid before inclusion in the peptide or peptide synthesis followed by chemical alkylation of the peptide. Without being held to any particular theory, it is believed that alkylation of peptides will achieve similar, if not the same, effects as acylation of the peptides, e.g., a prolonged half-life in circulation, a delayed onset of action, an extended duration of action, and an improved resistance to proteases, such as DPP-IV.

The term "identity" as used herein relates to the similarity between two or more sequences. Identity is measured by dividing the number of identical residues by the total number of residues and multiplying the product by 100 to achieve a percentage. Thus, two copies of exactly the same sequence have 100% identity, whereas two sequences that have amino acid deletions, additions, or substitutions relative to one another have a lower degree of identity. Those skilled in the art will recognize that several computer programs, such as those that employ algorithms such as BLAST (Basic Local Alignment Search Tool, Altschul et al. (1993) J. Mol. Biol. 215:403-410) are available for determining sequence identity.

As used herein, the term "selectivity" of a molecule for a first receptor relative to a second receptor refers to the comparison: Establishing a first ratio representing the EC50 of the test molecule divided by the EC50 of first receptor native hormone at the first receptor, followed by establishing a second ratio representing the EC50 of the test molecule at a second receptor divided by the EC50 of second receptor natural hormone, and comparing the first ratio to the second ratio. For example, a molecule that has a first ratio of two at a first receptor and second ratio of one at a second receptor has 2-fold selectivity for the first receptor relative to the second receptor.

As used herein, "glucagon potency" of a molecule refers to the ratio of the EC50 of the molecule at glucagon receptor divided by the EC50 of native glucagon at glucagon receptor.

As used herein, "GIP potency" of a molecule refers to the ratio of the EC50 of the molecule at GIP receptor divided by the EC50 of native GIP at GIP receptor.

As used herein, "GLP-1 potency" of a molecule refers to the ratio of the EC50 of the molecule at GLP-1 receptor divided by the EC50 of native GLP-1 at GLP-1 receptor.

As used herein, the term "alkyl" refers to a linear or branched hydrocarbon containing the indicated number of carbon atoms. Exemplary alkyls include methyl, ethyl, and linear propyl groups. The term "C₁-C_n alkyl" wherein n can be from 1 through 6, as used herein, represents a branched or linear alkyl group having from one to the specified number of carbon atoms. Typical C₁-C₆ alkyl groups include, but are not limited to, methyl, ethyl, n-propyl, iso-propyl, butyl, iso-Butyl, sec-butyl, tert-butyl, pentyl, hexyl and the like.

As used herein, the term "heteroalkyl" refers to a linear or branched hydrocarbon containing the indicated number of carbon atoms and at least one

heteroatom in the backbone of the structure. Suitable heteroatoms for purposes herein include but are not limited to N, S, and O.

As used herein, the term “cycloalkyl” refers to a cyclic hydrocarbon group containing the indicated number of carbon atoms, e.g., cyclopropyl, cyclobutyl,
5 cyclohexyl, and cyclopentyl.

As used herein, the term “heterocyclic” refers to a cyclic hydrocarbon group containing the indicated number of carbon atoms and one to three heteroatoms independently selected from the group consisting of oxygen, nitrogen, and sulfur. Nonlimiting examples of heterocycloalkyl groups include piperidine, tetrahydrofuran,
10 tetrahydropyran, dihydrofuran, morpholine, thiophene, and the like.

As used herein, the term “aryl” refers to a monocyclic or polycyclic aromatic group, preferably a monocyclic or bicyclic aromatic group, e.g., phenyl or naphthyl, containing the indicated number of carbon atoms. Unless otherwise indicated, an aryl group can be unsubstituted or substituted.

As used herein, the term “heteroaryl” refers to a monocyclic or polycyclic aromatic group containing the indicated number of carbon atoms and at least one heteroatom selected from the group consisting of oxygen, nitrogen, and sulfur. Unless otherwise indicated, an aryl group can be unsubstituted or substituted.

As used herein the inclusion of an “R” group at the carboxy terminus of a peptide with the designation that “‘R’ is COOH or CONH₂” designates that the C-terminal amino acid comprises a natural terminal carboxylic acid or an amide group in place of the natural terminal carboxylic acid.

ABBREVIATIONS:

25 Lower case k = D-isomer of lysine
Upper case K = L-isomer of lysine
γE = L-isomer of gamma, glutamic acid
(miniPEG)₂ = NH₂(CH₂CH₂O)₂CH₂COOH
COC₁₈H₃₂CO₂H = (C20 diacid)
30 (N-Me)G = sarcosine
Pip = piperidine-2-carboxylic acid
Aze = Azetidine-2-carboxylic acid
LysN3 = azido,lysine
βK = beta-lysine

aMe,L = alpha methylated Leucine

HyP = hydroxy proline

PGT= PropargylGlycine-Triazole

5 EMBODIMENTS

As disclosed herein, novel peptides exhibiting agonist activity at both the family of amylin receptors and the calcitonin receptor are provided. These peptides are referred to herein as DACRA (Dual Amylin Calcitonin Receptor Agonist) peptides. More particularly, analogs of amylin are provided having enhance
 10 pharmacological properties including enhanced stability, solubility and duration of activity while retaining activity at the amylin receptors, particularly amylin receptor 3. Amylin receptor 3 is associated with appetite suppression and agonists to amylin receptor 3 have therapeutic value as weight loss agents. Peptides having agonist activity at the amylin receptor are particularly advantageous for inducing weight loss
 15 or preventing weight gain, as well as for treating metabolic syndrome and diabetes, particularly when conjugated to an incretin. Thus, in one aspect, the invention provides methods for inducing weight loss or preventing weight gain, which involves administering to a patient in need thereof an effective amount of a peptide, that exhibits activity at the family of amylin receptors.

20 The novel DACRA peptides disclosed herein have high balanced potency at all the three amylin receptors and the calcitonin receptor where they function as balanced promiscuous agonists to deliver enhanced body weight lowering. The presently disclosed peptide analogs are more potent than cagrilintide, a drug development candidate DACRA. Prodrug derivatives of the novel DACRA peptides
 25 disclosed herein are also provided. These prodrug derivatives further extend and flatten the pharmacokinetics and dynamics of the DACRA peptides.

In accordance with one embodiment a peptide having amylin/calcitonin co-agonist activity (i.e. a DACRA peptide) is provided, wherein the peptide comprises the sequence of

30 $R_{40}-X_1CNTX_5TCAX_9QRLAEFLRHX_{19}SNNFGX_{25}IL-W_1-X_{28}X_{29}TNVGSNTZ-R_{20}$ (SEQ ID NO: 1) wherein

X_1 is Lys, Orn, beta-Orn or beta-Lys;

X_5 is Ala or Ser;

X_5 is Ala or Ser;

X₉ is Thr, isoacyl-Thr, N-acetyl-Thr, or isoacyl-Thr acylated at the alpha amine with a self-cleaving dipeptide element (A-B), wherein A is an amino acid and B is an N-alkylated amino acid;

W₁ is absent or comprises the structure NH₂(CH₂CH₂O)_m(CH₂)_nCOOH,
 5 wherein m is an integer selected from the range of 1-20 and n is 1 or 2, optionally wherein m is 2, 4, 6, 8 or 10 and n is 1 or 2, optionally wherein m is 8 and n is 2;

X₁₉ is Ser, N-acetyl-Ser or isoacyl-Ser acylated at the alpha amine with a self-cleaving dipeptide element (A-B), wherein A is an amino acid and B is an N-alkylated amino acid;

10 X₂₅ is Ala, Pro or hydroxyproline;

X₂₈ is Ser, Orn, Lys, hydroxyproline, N-acyl-hydroxy-Orn, N-acyl-hydroxy-Lys, N-acyl-hydroxyproline, or isoacyl-hydroxyproline acylated at the alpha amine with a self-cleaving dipeptide element (A-B), wherein A is an amino acid and B is an N-alkylated amino acid;

15 X₂₉ is Ser, Pro or hydroxyproline;

Z is hydroxyproline or N-acyl-hydroxyproline;

R₂₀ is COOH or CONH₂ and

R₄₀ is absent, or a C16-C30 fatty acid, a C16-C30 phosphonic fatty acid, a C16-C30 diacid or a C16-C30 alkyl covalently linked to the alpha amine, the
 20 beta amine, or the side chain amine of the amino acid at position 1 (X₁), optionally via a spacer; wherein said spacer is selected from the group consisting of a gamma glutamic acid, a gamma glutamic acid-gamma glutamic acid dipeptide, and a (gamma glutamic acid)_z-[COCH₂(OCH₂CH₂)_k-NH]_q-(gamma glutamic acid)_p, wherein z is 0 or 1, k is an integer selected from the range of 2-4 and q and p are independently an
 25 integer selected from the range of 0-4. In accordance with one embodiment X₁ is Lys, dLys, Orn, beta-Orn or beta-Lys acylated with a C16-C30 fatty acid, a C16-C30 phosphonic fatty acid, or a C16-C30 diacid. In one embodiment X₁ is beta-Lys, optionally acylated at the beta amine with a C16-C30 diacid, optionally via a linker. In one embodiment the peptide of SEQ ID NO: 1 is provided wherein X₁ is Lys, dLys,
 30 Orn, beta-Orn or beta-Lys acylated with γE-COC₁₈H₃₆CO₂H, X₂₅ is Ala, and Z is 4-hydroxyproline. In one embodiment the peptide of SEQ ID NO: 1 is provided wherein X₁ is Lys or beta-Lys acylated at the side chain amine with a C16-C30 fatty

acid, a C16-C30 phosphonic fatty acid, or a C16-C30 diacid. In a further embodiment X_1 is an amino acid in the D-stereoisomer configuration.

In one embodiment a peptide having amylin/calcitonin co-agonist activity is provided wherein the peptide comprises the sequence of

5 $R_{40}-X_1\text{CNTATCATQRLAEFLRHSSNNFGAILZSTNVGSNTZ-CONH}_2$
(SEQ ID NO: 3)

wherein

X_1 is Lys Orn, beta-Orn or beta-Lys;

Z is hydroxyproline or N-acyl-hydroxyproline;

10 and

R_{40} is a C16-C30 fatty acid, C16-C30 diacid or a C16-C30 alkyl covalently linked to the alpha amine, the beta amine or the side chain amine of the amino acid at position 1 (X_1), optionally via a spacer; wherein said spacer is selected from the group consisting of a gamma glutamic acid, a gamma glutamic acid-gamma glutamic acid dipeptide, and a (gamma glutamic acid) $_z$ -[COCH $_2$ (OCH $_2$ CH $_2$) $_k$ -NH] $_q$ -(gamma glutamic acid) $_p$, wherein z is 0 or 1, k is an integer selected from the range of 2-4 and q and p are independently an integer selected from the range of 0-4. In one embodiment the peptide of SEQ ID NO: 1 is provided wherein X_1 is beta-Lys, optionally in the D-stereoisomer configuration, Z is 4-hydroxyproline or 3-hydroxyproline. In a further embodiment a C16-C30 fatty acid, a C16-C30 phosphonic fatty acid, or a C16-C30 diacid is covalently linked to the alpha amine of Lys, or the beta amine of beta-Lys, present at position 1 of the peptide of SEQ ID NO: 1 via (gamma glutamic acid) $_z$ -[COCH $_2$ (OCH $_2$ CH $_2$) $_k$ -NH] $_q$ -(gamma glutamic acid) $_p$ spacer, wherein z is 0 or 1, k is an integer selected from the range of 2-4 and q and p are independently an integer selected from the range of 0-4. In one embodiment the spacer is $\gamma\text{E-COC}_{18}\text{H}_{36}\text{CO}_2\text{H}$.

In accordance with one embodiment the peptide of SEQ ID NO: 1, SEQ ID NO: 2 or SEQ ID NO: 3 is provided wherein X_1 is beta-Lys, optionally in the D-stereoisomer configuration, wherein a C16-C22 fatty acid, a C16-C30 phosphonic fatty acid, or a C16-C22 diacid is covalently linked to the beta amine of beta-Lys via a spacer having the structure:

-(gamma glutamic acid) $_p$ -; or -[COCH $_2$ (OCH $_2$ CH $_2$) $_k$ NH] $_q$ -(gamma glutamic acid) $_p$ -; wherein k is 2, p is 1 or 2 and q is an integer selected from 1, 2 or 4, further

wherein Z is 4-hydroxyproline or 3- hydroxyproline and R₂₀ is CONH₂. In a further embodiment Z is 4-hydroxyproline and R₄₀ is γ E-COC₁₈H₃₆CO₂H.

In accordance with one embodiment, a prodrug isoacyl peptide derivative of any of the DACRA peptides disclosed herein is provided. The combined action of the prodrug and its conversion to the drug yields an extended action, high potency co-agonist where the plasma concentration is tightly controlled to maximize body weight reduction with much lessened risk of GI toxicity. In one embodiment the prodrug comprises an ester bond between any two adjacent amino acids of SEQ ID NO: 1, optionally wherein the ester bond is stabilized by the covalent linkage of a self-cleaving dipeptide prodrug element linked via an amide bond to the alpha amine of one or more isoacyl amino acids present in the prodrug DACRA analog.

In one embodiment any of the DACRA peptides disclosed herein is modified to comprise an isoacyl-Thr at position 9 of said peptide, relative to the amino acid sequence of SEQ ID NO: 1, thus introducing an ester bond between the amino acids at position 8 and 9. Such isoacyl peptide derivatives are inactive at the amylin and calcitonin receptors. A subsequent O to N acyl shift (reconstituting the amide bond between amino acids at positions 8 and 9) restores the amylin/calcitonin receptor activity to the DACRA peptide. The timing of the O to N acyl shift can be regulated by the attachment of a self-cleaving dipeptide prodrug element linked to the alpha amine of the isoacyl amino acid. Attachment of the dipeptide prodrug element prevents the O to N acyl shift until the dipeptide element self cleaves from the peptide and allows the O to N acyl shift to proceed, and thus restore amylin/calcitonin receptor activity to the peptide. The speed of the chemical cleavage of the dipeptide prodrug element is a function of the specific prodrug element sequence. Thus, by selecting the amino acids comprising the dipeptide element the timing of the conversion from prodrug to active form can be controlled to extend the duration of action of the DACRA peptide.

In one embodiment the alpha amine of an isoacyl-Thr located at position 9 of the DACRA peptides of the present invention is covalently linked via an amide bond to a dipeptide element (A-B), wherein A is an amino acid, and B is an N-alkylated amino acid. The rate at which the dipeptide prodrug element will chemically cleave is determined based on the side chains of A and B.

In some embodiments A and B are selected to inhibit enzymatic cleavage of the A-B dipeptide from the DACRA peptide by enzymes found in mammalian serum.

In some embodiments A and/or B are selected such that the cleavage of A-B from the DACRA peptide in PBS under physiological conditions, is not more than two fold the cleavage half-life of A-B from the DARA peptide in a solution comprising a DPP-IV protease (i.e., cleavage of A-B from the DACRA peptide does not occur at a rate more
5 than 2x faster in the presence of DPP-IV protease and physiological conditions relative to identical conditions in the absence of the enzyme). In some embodiments A and/or B is an amino acid in the D stereoisomer configuration. In some exemplary embodiments, A is an amino acid in the D stereoisomer configuration and B is an amino acid in the L stereoisomer configuration. In some exemplary embodiments, A
10 is an amino acid in the L stereoisomer configuration and B is an amino acid in the D stereoisomer configuration. In some exemplary embodiments, A is an amino acid in the D stereoisomer configuration and B is an amino acid in the D stereoisomer configuration.

As disclosed herein prodrugs of the DACRA peptide of the present disclosure
15 are highly effective in lowering body weight in obese rodents. These peptides demonstrate a much-sustained duration of pharmacological action that should serve to minimize frequency in human dosing. They also demonstrate a much-reduced peak to trough concentration between injections which supports more precise dosing to achieve maximal efficacy (weight lowering) and minimal toxicity (nausea, flatulence,
20 and vomiting). The chemical form of the novel peptides is additionally supportive of non-injectable forms of administration, most notably oral and inhalation.

In one aspect, the invention provides methods for inducing weight loss or preventing weight gain, which involve administering to a patient in need thereof an effective amount of a peptide, that exhibits activity at amylin receptor 3, optionally in
25 the form of a prodrug.

In accordance with one embodiment an isoacyl peptide derivative of SEQ ID NO: 1 or SEQ ID NO: 1 is provided wherein an ester bond is formed between the amino acids at position 8 and 9. In one embodiment the amino acid at position 9 is serine or threonine. In one embodiment the amino acid at position 9 is an isoacyl-
30 threonine, wherein the ester bond is formed between the side chain hydroxyl group of threonine and the alpha carboxylic acid group of the amino acid at position 8, optionally wherein the amino acid at position 8 is alanine or serine.

In accordance with one embodiment a prodrug derivative of any of the DACRA peptides disclosed herein is provided. In one embodiment the DACRA

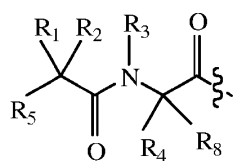
peptides of SEQ ID NO: 1, SEQ ID NO: 2 or SEQ ID NO: 3 is provided, wherein said peptide is modified by the covalent linkage of one or more dipeptides (A-B) to an aliphatic primary amine of the peptide, wherein A is an amino acid or a hydroxy acid and B is an N-alkylated amino acid linked to said peptide through an amide bond

5 between a carboxyl moiety of B and an amine of the peptide, wherein the side chain of the first amino acid (A) of said dipeptide is acylated with a C16-C30 fatty acid, a C16-C30 phosphonic fatty acid, or a C16-C30 diacid. In accordance with one embodiment, a dipeptide prodrug element is covalently linked to an isoacyl peptide analog wherein the DACRA peptide of SEQ ID NO: 1, SEQ ID NO: 2 or SEQ ID

10 NO: 1 is modified to comprise an isoacyl amino acid in the peptide, optionally at a position selected from positions 9, 19 and 28 relative to SEQ ID NO: 1. In one embodiment a dipeptide prodrug element is covalently linked to an isoacyl amino acid at position 9 of SEQ ID NO: 1 or SEQ ID NO: 2, optionally wherein the isoacyl amino acid is isoacyl-Thr and the dipeptide element is linked to the alpha amine of the

15 isoacyl-Thr.

In accordance with one embodiment the dipeptide prodrug element comprises the structure:



I

wherein

(I) R₁ and R₂ are independently selected from the group consisting of H, deuterium, C₁-C₁₈ alkyl, C₂-C₁₈ alkenyl, (C₁-C₁₈ alkyl)OH, (C₁-C₁₈ alkyl)SH, (C₂-C₃ alkyl)SCH₃, (C₁-C₄ alkyl)CONH₂, (C₁-C₄ alkyl)COOH, (C₁-C₄ alkyl)NH₂, (C₁-C₄ alkyl)NHC(NH₂⁺)NH₂, (C₀-C₄ alkyl)(C₃-C₆ cycloalkyl), (C₀-C₄ alkyl)(C₂-C₅ heterocyclic), (C₀-C₄ alkyl)(C₆-C₁₀ aryl)R₇, (C₁-C₄ alkyl)(C₃-C₉ heteroaryl), and C₁-C₁₂ alkyl(W₁₀)C₁-C₁₂ alkyl, wherein W₁₀ is a heteroatom selected from the group consisting of N, S and O, or R₁ and R₂ together with the atoms to which they are

20 attached form a C₃-C₁₂ cycloalkyl;

R₃ is CD₃, C₁-C₁₈ alkyl;

R₄ and R₈ are each H or D;

R₅ is NHR₆, or R₅ and R₂ together with the atoms to which they are attached form a 4, 5 or 6 member heterocyclic ring;

R₆ is H or C₁-C₄ alkyl; and,

R₇ is selected from the group consisting of H, OH, halo, (C₁-C₇ alkyl), (C₂-C₇ alkenyl), OCF₃, NO₂, CN, NC, O(C₁-C₇ alkyl), CO₂H, CO₂(C₁-C₇ alkyl), NHR₆, aryl, and heteroaryl; or

(II) R₁ and R₂ are independently selected from the group consisting of H, C₁-C₁₈ alkyl, C₂-C₁₈ alkenyl, (C₁-C₁₈ alkyl)OH, (C₁-C₁₈ alkyl)SH, (C₂-C₃ alkyl)SCH₃, (C₁-C₄ alkyl)CONH₂, (C₁-C₄ alkyl)COOH, (C₁-C₄ alkyl)NH₂, (C₁-C₄ alkyl)NHC(NH₂⁺)NH₂, (C₀-C₄ alkyl)(C₃-C₆ cycloalkyl), (C₀-C₄ alkyl)(C₂-C₅ heterocyclic), (C₀-C₄ alkyl)(C₆-C₁₀ aryl)R₇, (C₁-C₄ alkyl)(C₃-C₉ heteroaryl), and C₁-C₁₂ alkyl(W₁₀)C₁-C₁₂ alkyl, wherein W₁₀ is a heteroatom selected from the group consisting of N, S and O, or R₁ and R₂ together with the atoms to which they are attached form a C₃-C₁₂ cycloalkyl;

R₃ is C₁-C₁₈ alkyl;

R₄ is selected from the group consisting of CH₃, CH₂(C₁-C₁₀ alkyl), CH₂(C₂-C₁₀ alkenyl), CH₂(C₀-C₁₀ alkyl)OH, CH₂(C₀-C₁₀ alkyl)SH, CH₂(C₀-C₃ alkyl)SCH₃, CH₂(C₀-C₃ alkyl)CONH₂, CH₂(C₀-C₃ alkyl)COOH, CH₂(C₀-C₃ alkyl)NH₂, CH₂(C₀-C₃ alkyl)NHC(NH₂⁺)NH₂, CH₂(C₀-C₃ alkyl)(C₃-C₆ cycloalkyl), CH₂(C₀-C₃ alkyl)(C₂-C₅ heterocyclic), CH₂(C₀-C₃ alkyl)(C₆-C₁₀ aryl)R₇, CH₂(C₁-C₃ alkyl)(C₃-C₉ heteroaryl), and CH₂(C₀-C₁₂ alkyl)(W₁₀)C₁-C₁₂ alkyl, wherein W₁₀ is a heteroatom selected from the group consisting of N, S and O, or R₄ and R₃ together with the atoms to which they are attached form a pyrrolidine, a 3,4-dehydropyrrolidine, a hydroxypyrrolidine, a piperidine or a hydroxypiperidine ring;

R₈ is H;

R₅ is NHR₆, or R₅ and R₂ together with the atoms to which they are attached form a 4, 5 or 6 member heterocyclic ring;

R₆ is H or C₁-C₄ alkyl; and,

R₇ is selected from the group consisting of H, OH, halo, (C₁-C₇ alkyl), (C₂-C₇ alkenyl), OCF₃, NO₂, CN, NC, O(C₁-C₇ alkyl), CO₂H, CO₂(C₁-C₇ alkyl), NHR₆, aryl, and heteroaryl; or

(III) R₁ and R₂ are independently selected from the group consisting of H, C₁-C₁₈ alkyl, C₂-C₁₈ alkenyl, (C₁-C₁₈ alkyl)OH, (C₁-C₁₈ alkyl)SH, (C₂-C₃ alkyl)SCH₃,

(C₁-C₄ alkyl)CONH₂, (C₁-C₄ alkyl)COOH, (C₁-C₄ alkyl)NH₂, (C₁-C₄ alkyl)NHC(NH₂⁺)NH₂, (C₀-C₄ alkyl)(C₃-C₆ cycloalkyl), (C₀-C₄ alkyl)(C₂-C₅ heterocyclic), (C₀-C₄ alkyl)(C₆-C₁₀ aryl)R₇, (C₁-C₄ alkyl)(C₃-C₉ heteroaryl), and C₁-C₁₂ alkyl(W₁₀)C₁-C₁₂ alkyl, wherein W₁₀ is a heteroatom selected from the group consisting of N, S and O, or R₁ and R₂ together with the atoms to which they are attached form a C₃-C₁₂ cycloalkyl; or R₁ and R₂ together with the atoms to which they are attached form a C₃-C₁₂ cycloalkyl;

R₃ is C₁-C₁₈ alkyl;

R₄ is independently selected from the group consisting of CH(C₁-C₈ alkyl)₂, CH(C₂-C₈ alkenyl)₂, CH(C₁-C₈ alkyl)(OH), CH(C₁-C₈ alkyl)((C₁-C₈ alkyl)SH), and CH(C₁-C₃ alkyl)((C₁-C₈ alkyl)(NH₂)), or R₄ and R₃ together with the atoms to which they are attached form a pyrrolidine, a 3,4-dehydropyrrolidine, a hydroxypyrrolidine, a piperidine or a hydroxypiperidine ring;

R₈ is H;

R₅ is NHR₆, or R₅ and R₂ together with the atoms to which they are attached form a 4, 5 or 6 member heterocyclic ring;

R₆ is H or C₁-C₄ alkyl; and,

R₇ is selected from the group consisting of H, OH, halo, (C₁-C₇ alkyl), (C₂-C₇ alkenyl), OCF₃, NO₂, CN, NC, O(C₁-C₇ alkyl), CO₂H, CO₂(C₁-C₇ alkyl), NHR₆, aryl, and heteroaryl; or

(IV) R₁ and R₂ are independently selected from the group consisting of H, C₁-C₁₈ alkyl, C₂-C₁₈ alkenyl, (C₁-C₁₈ alkyl)OH, (C₁-C₁₈ alkyl)SH, (C₂-C₃ alkyl)SCH₃, (C₁-C₄ alkyl)CONH₂, (C₁-C₄ alkyl)COOH, (C₁-C₄ alkyl)NH₂, (C₁-C₄ alkyl)NHC(NH₂⁺)NH₂, (C₀-C₄ alkyl)(C₃-C₆ cycloalkyl), (C₀-C₄ alkyl)(C₂-C₅ heterocyclic), (C₀-C₄ alkyl)(C₆-C₁₀ aryl)R₇, (C₁-C₄ alkyl)(C₃-C₉ heteroaryl), and C₁-C₁₂ alkyl(W₁₀)C₁-C₁₂ alkyl, wherein W₁₀ is a heteroatom selected from the group consisting of N, S and O, or R₁ and R₂ together with the atoms to which they are attached form a C₃-C₁₂ cycloalkyl;

R₃ is CD₃, C₁-C₁₈ alkyl;

R₄ and R₈ are each H or D;

R₅ is NHR₆, or R₅ and R₂ together with the atoms to which they are attached form a 4, 5 or 6 member heterocyclic ring;

R₆ is H or C₁-C₄ alkyl; and,

R₇ is selected from the group consisting of H, OH, halo, (C₁-C₇ alkyl), (C₂-C₇ alkenyl), OCF₃, NO₂, CN, NC, O(C₁-C₇ alkyl), CO₂H, CO₂(C₁-C₇ alkyl), NHR₆, aryl, and heteroaryl; or

(V) R₁ and R₂ are independently selected from the group consisting of H, C₁-C₁₈ alkyl, C₂-C₁₈ alkenyl, (C₁-C₁₈ alkyl)OH, (C₁-C₁₈ alkyl)SH, (C₂-C₃ alkyl)SCH₃, (C₁-C₄ alkyl)CONH₂, (C₁-C₄ alkyl)COOH, (C₁-C₄ alkyl)NH₂, (C₁-C₄ alkyl)NHC(NH₂⁺)NH₂, (C₀-C₄ alkyl)(C₃-C₆ cycloalkyl), (C₀-C₄ alkyl)(C₂-C₅ heterocyclic), (C₀-C₄ alkyl)(C₆-C₁₀ aryl)R₇, (C₁-C₄ alkyl)(C₃-C₉ heteroaryl), and C₁-C₁₂ alkyl(W₁₀)C₁-C₁₂ alkyl, wherein W₁₀ is a heteroatom selected from the group consisting of N, S and O, or R₁ and R₂ together with the atoms to which they are attached form a C₃-C₁₂ cycloalkyl; or R₁ and R₂ together with the atoms to which they are attached form a C₃-C₁₂ cycloalkyl;

10 R₃ is C₁-C₁₈ alkyl;

R₄ is independently selected from the group consisting of CH(C₁-C₈ alkyl)₂, CH(C₂-C₈ alkenyl)₂, CH(C₁-C₈ alkyl)(OH), CH(C₁-C₈ alkyl)((C₁-C₈ alkyl)SH), and CH(C₁-C₃ alkyl)((C₁-C₈ alkyl)(NH₂)) or R₄ and R₃ together with the atoms to which they are attached form a pyrrolidine, a 3,4-dehydropyrrolidine, a hydroxypyrrolidine, a piperidine or a hydroxypiperidine ring;

R₈ is H;

R₅ is NHR₆, or R₅ and R₂ together with the atoms to which they are attached form a 4, 5 or 6 member heterocyclic ring;

R₆ is H or C₁-C₄ alkyl; and,

R₇ is selected from the group consisting of H, OH, halo, (C₁-C₇ alkyl), (C₂-C₇ alkenyl), OCF₃, NO₂, CN, NC, O(C₁-C₇ alkyl), CO₂H, CO₂(C₁-C₇ alkyl), NHR₆, aryl, and heteroaryl; or

20 (VI) R₁ and R₂ are independently selected from the group consisting of H, C₁-C₁₈ alkyl, C₂-C₁₈ alkenyl, (C₁-C₁₈ alkyl)OH, (C₁-C₁₈ alkyl)SH, (C₂-C₃ alkyl)SCH₃, (C₁-C₄ alkyl)CONH₂, (C₁-C₄ alkyl)COOH, (C₁-C₄ alkyl)NH₂, (C₁-C₄ alkyl)NHC(NH₂⁺)NH₂, (C₀-C₄ alkyl)(C₃-C₆ cycloalkyl), (C₀-C₄ alkyl)(C₂-C₅ heterocyclic), (C₀-C₄ alkyl)(C₆-C₁₀ aryl)R₇, (C₁-C₄ alkyl)(C₃-C₉ heteroaryl), and C₁-C₁₂ alkyl(W₁₀)C₁-C₁₂ alkyl, wherein W₁₀ is a heteroatom selected from the group consisting of N, S and O, or R₁ and R₂ together with the atoms to which they are

attached form a C₃-C₁₂ cycloalkyl; or R₁ and R₂ together with the atoms to which they are attached form a C₃-C₁₂ cycloalkyl;

R₃ is C₁-C₁₈ alkyl;

R₄ is independently selected from the group consisting of CH(C₁-C₈ alkyl)₂,
 5 CH (C₂-C₈ alkenyl)₂, CH(C₁-C₈ alkyl)(OH), CH(C₁-C₈ alkyl)((C₁-C₈ alkyl)SH), and
 CH(C₁-C₃ alkyl)((C₁-C₈ alkyl)(NH₂)) or R₄ and R₃ together with the atoms to which
 they are attached form a pyrrolidine, a 3,4-dehydropyrrolidine, a hydroxypyrrolidine,
 a piperidine or a hydroxypiperidine ring;

R₈ is H;

10 R₅ is NHR₆, or R₅ and R₂ together with the atoms to which they are attached
 form a 4, 5 or 6 member heterocyclic ring;

R₆ is H or C₁-C₄ alkyl; and,

R₇ is selected from the group consisting of H, OH, halo, (C₁-C₇ alkyl), (C₂-C₇
 alkenyl), OCF₃, NO₂, CN, NC, O(C₁-C₇ alkyl), CO₂H, CO₂(C₁-C₇ alkyl), NHR₆, aryl,
 and heteroaryl; or

(VII) R₁ is (C₁-C₄ alkyl)NH₂, optionally lLys or dLys;

R₂, R₈ are both H;

15 R₄ and R₃ together with the atoms to which they are attached form a
 pyrrolidine, a 3,4-dehydropyrrolidine, a hydroxypyrrolidine, a piperidine or a
 hydroxypiperidine ring; and

R₅ is NH₂., optionally wherein R₄ and R₃ together with the atoms to which
 they are attached form a hydroxy substituted pyrrolidine ring or a piperidine ring, or

20

(VIII) R₁ is (C₁-C₄ alkyl)NH₂, optionally lLys or dLys, acylated with a C16-
 C20 fatty acid, a C16-C20 phosphonic fatty acid, or a C16-C20 diacid;

R₂ is H;

R₄ and R₈ are both D;

25

R₃ is CD₃; and

R₅ is NH₂; or

(IX) R₁ is (C₁-C₄ alkyl)NH₂, optionally lLys or dLys, acylated with a C16-
 C20 fatty acid, a C16-C20 phosphonic fatty acid, or a C16-C20 diacid;

R₂, R₄ and R₈ are each H;

30

R₃ is CH₃; and

R₅ is NH₂.

In one embodiment the prodrug element having the structure of Formula I and the substituents of any of I-VII is further modified by acylation of the side chain of the first amino acid of dipeptide element A-B. More particularly, the first amino acid of dipeptide element A-B is acylated with a C16-C30 fatty acid, a C16-C30 phosphonic fatty acid, or a C16-C30 diacid, optionally via a dipeptide element spacer selected from the group consisting of a gamma glutamic acid, a gamma glutamic acid-gamma glutamic acid dipeptide, and a (gamma glutamic acid)_z-[COCH₂(OCH₂CH₂)_k-NH]_q-(gamma glutamic acid)_p, wherein z is 0 or 1, k is an integer selected from the range of 2-4 and q and p are independently an integer selected from the range of 0-4.

Acylation and alkylation

The DACRA peptides of the present invention are acylated or alkylated at the N-terminal alpha amine of the peptide and optionally additional sites of the peptide such as at the side chain amine of an amino acid (e.g., Lys or Orn) present at position 28 and/or at the first amino acid of the dipeptide prodrug element of the prodrug derivative of the DACRA peptides. Acylation or alkylation can increase the half-life of the DACRA peptides in circulation. Acylation or alkylation can advantageously delay the onset of action and/or extend the duration of action at the amylin receptors and/or improve resistance to proteases such as DPP-IV and/or improve solubility. Activity at the amylin and calcitonin receptors by DACRA peptides may be extended by acylation. In some embodiments, the potency of the acylated DACRA peptides is comparable to the unacylated versions of the glucagon peptides. In alternative embodiments, the potency of the acylated DACRA peptides is increased as compared to that of the unacylated version of the DACRA peptides.

In some embodiments, the DACRA peptides may further comprise a spacer between the amino acid at position 1 (or at amino acid A of the dipeptide prodrug element) of the DACRA peptide and the acyl group or alkyl group. In some embodiments, the acyl group is a fatty acid, a C16-C30 phosphonic fatty acid, or a diacid, or salt thereof, e.g. a C4 to C30 fatty acid/diacid, a C8 to C24 fatty acid/diacid, cholic acid, a C4 to C30 alkyl, a C8 to C24 alkyl, or an alkyl comprising a steroid moiety of a bile acid. The spacer is any moiety with suitable reactive groups for

attaching acyl or alkyl groups. In exemplary embodiments, the spacer comprises an amino acid, a dipeptide, a tripeptide, a hydrophilic bifunctional, or a hydrophobic bifunctional spacer. In some embodiments, the spacer is selected from the group consisting of: Trp, Glu, Asp, Cys and a spacer comprising

- 5 $\text{NH}_2(\text{CH}_2\text{CH}_2\text{O})_n(\text{CH}_2)_m\text{COOH}$, wherein m is any integer from 1 to 6 and n is any integer from 2 to 12. Such acylated or alkylated glucagon peptides may also further comprise a hydrophilic moiety, optionally a polyethylene glycol. Any of the foregoing DACRA peptides may comprise two acyl groups or two alkyl groups, or a combination thereof.

- 10 The acyl group of the acylated DACRA peptides can be of any size, e.g., any length carbon chain, and can be linear or branched. In some specific embodiments of the invention, the acyl group is a C8 to C30 fatty acid, a C16-C30 phosphonic fatty acid, or a C8 to C30 diacid. For example, the acyl group can be any of a C8 fatty acid/phosphonic fatty acid/diacid, C10 fatty acid/phosphonic fatty acid/diacid, C12
15 fatty acid/phosphonic fatty acid/diacid, C14 fatty acid/phosphonic fatty acid/diacid, C16 fatty acid/phosphonic fatty acid/diacid, C18 fatty acid/phosphonic fatty acid/diacid, C20 fatty acid/phosphonic fatty acid/diacid, C22 fatty acid/phosphonic fatty acid/diacid, C24 fatty acid/phosphonic fatty acid/diacid, C26 fatty acid/phosphonic fatty acid/diacid, C28 fatty acid/phosphonic fatty acid/diacid, or a
20 C30 fatty acid/phosphonic fatty acid/diacid. In some embodiments, the acyl group is a C8 to C20 fatty acid/phosphonic fatty acid/diacid, e.g., a C18 fatty acid/diacid or a C20 fatty acid/phosphonic fatty acid/diacid.

Addition modifications can be made to the co-agonist peptides of SEQ ID NO: 1, SEQ ID NO: 2 and SEQ ID NO: 1, including but are not limited to:

- 25 (A) Improving solubility, for example, by introducing one, two, three or more charged amino acid(s) to the C-terminal portion of the peptide, preferably at the carboxy terminus of the DACRA peptide.

- (B) Increasing solubility and duration of action or half-life in circulation by the addition of a hydrophilic moiety such as a polyethylene glycol chain, e.g. at
30 position 28, or 29, within a linker joining amino acids 28 and 29, or at the C-terminal amino acid of the peptide,

(C) Non-conservative or conservative substitutions that do not substantially affect activity, for example, conservative substitutions at one or more of positions, 27, 28 or 29.

The applicant has discovered that removing the C-terminal 10-12 amino acids of the peptide of SEQ ID NO: 1 produced a N-terminal fragment of the DACRA peptides that exhibits reduced activity at the amylin receptor but retains full activity at the calcitonin receptor. Accordingly, a C-terminal truncated DACRA peptide,
 5 missing 1 to 10 amino acids from the C-terminus, including for example the peptides of SEQ ID NO: 5 or SEQ ID NO: 2, have utility as calcitonin selective DACRA analogs. The lower activity of these peptides at the amylin receptor allows higher doses to be administered to induce bone growth or prevent bone loss in patients in need of such therapy while minimizing adverse gastrointestinal impacts.

10 Applicants have thus discovered that regions of the DACRA peptides function simply as a linker and are not critical for activity at the amylin receptor (see Figs. 11-18). Accordingly, DACRA analogs can be prepared comprising a linker located between amino acids 27 and 28 while retaining activity at the amylin receptor. In one embodiment a DACRA analog is provided comprising a linker joining amino acids 1-
 15 27 to amino acids 28-37, wherein the linker comprises one or more functional groups allowing for the attachment of other bioactive molecules to form DACRA conjugates. In one embodiment the DACRA conjugate comprises an incretin peptide covalently bound to the linker joining the DACRA amino acids 1-27 to amino acids 28-37 or 29-37. In one embodiment a DACRA analog is provided comprising a linker joining
 20 amino acids 1-27 to amino acids 28-37, wherein the linker comprises the structure, $[\text{NH}_2(\text{CH}_2\text{CH}_2\text{O})_2\text{CH}_2\text{COOH}]$ or $[\text{NH}_2(\text{CH}_2\text{CH}_2\text{O})_m\text{CH}_2\text{CH}_2\text{COOH}]$, wherein m is 4-10, optionally 4, 6, 8 or 10, optionally wherein m is 8.

In accordance with one embodiment a modified DACRA peptide suitable for conjugation with another bioactive compound is provided wherein the modified
 25 DACRA peptide comprises the structure:

$\text{R}_{40}\text{-X}_1\text{CNTATCATQRLAEFLRHSSNNFGAIL-W}_1\text{-X}_{28}\text{STNVGSNTX}_{37}\text{-CONH}_2$ (SEQ ID NO: 4) wherein

X_1 is Lys or beta-Lys;

30 X_{28} is Orn or Lys, said Orn or Lys being linked to W_1 via the side chain amine of Orn or Lys;

X_{37} is hydroxyproline or proline;

W_1 is a linker having the structure $[\text{NH}_2(\text{CH}_2\text{CH}_2\text{O})_2\text{CH}_2\text{COOH}]$ or $[\text{NH}_2(\text{CH}_2\text{CH}_2\text{O})_m\text{CH}_2\text{CH}_2\text{COOH}]$ wherein m is an integer selected from the range of

1-20, optionally wherein m is 8 or 10, optionally wherein W_1 is

$NH_2(CH_2CH_2O)_8CH_2CH_2COOH$; and

R_{40} is a C16-C30 fatty acid, C16-C30 diacid or a C16-C30 alkyl

covalently linked to the alpha amine of Lys, the beta amine of beta-Lys or the side

5 chain amine of the amino acid present at position 1, optionally via a spacer; wherein

said spacer is selected from the group consisting of a gamma glutamic acid, a gamma

glutamic acid-gamma glutamic acid dipeptide, and a (gamma glutamic acid) $_z$ -

$[COCH_2(OCH_2CH_2)_k-NH]_q$ -(gamma glutamic acid) $_p$, wherein z is 0 or 1, k is an

integer selected from the range of 2-4 and q and p are independently an integer

10 selected from the range of 0-4, optionally wherein R_{40} is $\gamma E-COC_{18}H_{36}CO_2H$. In one

embodiment the peptide of SEQ ID NO: 4 is provided wherein X_{37} is hydroxyproline.

In one embodiment the peptide of SEQ ID NO: 4 is provided wherein X_1 is beta-Lys,

optionally in the D-stereoisomer configuration, and X_{37} is 4-hydroxyproline or 3-

hydroxyproline. In a further embodiment a C16-C30 fatty acid, a C16-C30

15 phosphonic fatty acid, or a C16-C30 diacid is covalently linked to the alpha amine of

Lys, or the beta amine of beta-Lys, present at position 1 of the peptide of SEQ ID NO:

1 via (gamma glutamic acid) $_z$ - $[COCH_2(OCH_2CH_2)_k-NH]_q$ -(gamma glutamic acid) $_p$

spacer, wherein z is 0 or 1, k is an integer selected from the range of 2-4 and q and p

are independently an integer selected from the range of 0-4, optionally wherein R_{40} is

20 $\gamma E-COC_{18}H_{36}CO_2H$.

In one embodiment the peptide of SEQ ID NO: 4 is provided wherein

W_1 is $NH_2(CH_2CH_2O)_2CH_2COOH$ or $NH_2(CH_2CH_2O)_mCH_2CH_2COOH$

wherein m is an integer selected from the range of 1-20, optionally wherein m is 8 or

10; and

25 R_{40} is a C16-C30 fatty acid, a C16-C30 phosphonic fatty acid, or a

C16-C30 diacid covalently linked to the alpha amine of Lys or the beta amine of beta-

Lys present at position 1 via a spacer having the structure (gamma glutamic acid) $_z$ -

$[COCH_2(OCH_2CH_2)_k-NH]_q$ -(gamma glutamic acid) $_p$, wherein z is 0 or 1, k is an

integer selected from the range of 2-4 and q and p are independently an integer

30 selected from the range of 0-4.

In one embodiment the peptide of SEQ ID NO: 4 is provided wherein

X_1 is beta-Lys, optionally in the D stereoisomer configuration;

X_{28} is Orn linked to W_1 via the side chain amine of Orn;

X_{37} is hydroxyproline;

W_1 is $NH_2(CH_2CH_2O)_m(CH_2)_nCOOH$, wherein m is an integer selected from 8 or 10 and n is 1 or 2, optionally wherein m is 8 and n is 2; and

R_{40} is a C16-C30 fatty acid, a C16-C30 phosphonic fatty acid, or a C16-C30 diacid covalently linked to the alpha amine of Lys or the beta amine of beta-Lys present at position 1 via a spacer having the structure (gamma glutamic acid) $_z$ -[COCH $_2$ (OCH $_2$ CH $_2$) $_k$ -NH] $_q$ -(gamma glutamic acid) $_p$, wherein z is 0 or 1, k is an integer selected from the range of 2-4 and q and p are independently an integer selected from the range of 0-4, optionally wherein R_{40} is $\gamma E-COC_{18}H_{36}CO_2H$.

In accordance with one embodiment, a modified DACRA peptide is provided, comprising the structure of

$R_{40}-X_1CNTATCATQRLAEFLRHSSNNFGAIL-W_1-X_{28}STNVGSNTX_{37}-CONH_2$ (SEQ ID NO: 4)

wherein

X_1 is Lys or beta-Lys, optionally where the amino acid at X_1 is in the D conformation;

X_{28} is Lys or Orn linked to W_1 via the side chain amine of the amino acid at position 28;

W_1 comprises a bifunctional linker having the structure

$NH_2(CH_2CH_2O)_m(CH_2)_nCOOH$, wherein m is an integer selected from the range of 1-20 and n is 1 or 2, optionally wherein m is 2, 4, 6, 8, or 10 and n is 1 or 2, optionally wherein W_1 comprises $NH_2(CH_2CH_2O)_8CH_2CH_2COOH$; and

R_{40} is absent, a C16-C30 fatty acid, a C16-C30 phosphonic fatty acid, a C16-C30 diacid group or a C16-C30 alkyl group covalently linked to the alpha amine of Lys or the beta amine of beta-Lys, optionally via a spacer; wherein said spacer is selected from the group consisting of a gamma glutamic acid, a gamma glutamic acid-gamma glutamic acid dipeptide, and a (gamma glutamic acid) $_z$ -[COCH $_2$ (OCH $_2$ CH $_2$) $_k$ -NH] $_q$ -(gamma glutamic acid) $_p$, wherein z is 0 or 1, k is an integer selected from the range of 2-4 and q and p are independently an integer selected from the range of 0-4, optionally wherein R_{40} is $\gamma E-COC_{18}H_{36}CO_2H$.

In accordance with one embodiment, a modified DACRA peptide of SEQ ID NO: 4 is provided wherein R_{40} is a C16-C30 fatty acid, C16-C30 diacid group or a C16-C30 alkyl group covalently linked to the N-terminal alpha amine or the beta

amine, optionally via a spacer; wherein said spacer is selected from the group consisting of a gamma glutamic acid, a gamma glutamic acid-gamma glutamic acid dipeptide, and a (gamma glutamic acid)_z-[COCH₂(OCH₂CH₂)_k-NH]_q-(gamma glutamic acid)_p, wherein z is 0 or 1, k is an integer selected from the range of 2-4 and
 5 q and p are independently an integer selected from the range of 0-4.

In a further embodiment the modified DACRA peptides of SEQ ID NO: 4 and 6 are provided wherein W₁ comprises the structure NH₂(CH₂CH₂O)_m(CH₂)_nCOOH, wherein m is an integer selected from the range of 1-20 and n is 1 or 2, optionally wherein m is 8 and n is 2.

10 In one embodiment the modified DACRA peptides disclosed herein are conjugated with another bioactive compound, including for example an incretin peptide, to either the linker W₁ or to the amino acid at position 28. In one embodiment the peptide of SEQ ID NO: 4 or 6 is provided wherein X₂₈ is Orn or Lys wherein an incretin peptide is covalently bound to the alpha amine of the Orn or Lys
 15 present at position 28, optionally via a linker having the structure of NH₂(CH₂CH₂O)_mCH₂COOH or NH₂(CH₂CH₂O)_mCH₂CH₂COOH, wherein m is an integer selected from the range of 1-20, optionally wherein m is 2, 4, 6, 8 or 10. In accordance with one embodiment a bioactive molecule is conjugated to the peptide of SEQ ID NO: 4 or 6 via a linker comprising the structure
 20 [NH₂(CH₂CH₂O)_m(CH₂)_nCOOH] wherein m is an integer selected from the range of 1-20 and n is 1 or 2, optionally wherein m is 8 and n is 2.

In accordance with one embodiment a DACRA/incretin conjugate is provided wherein the incretin peptide is selected from the group consisting of Semaglutide, tirzepatide, retatrutide and analogs thereof, wherein said incretins retain agonist
 25 activity at the GLP-1 receptor, and optionally at both the GIP and the GLP-1 receptors after conjugation to the DACRA peptide.

Pharmaceutical compositions comprising the DACRA peptides, DACRA conjugates or DACRA prodrug derivatives disclosed herein can be formulated and administered to patients using standard pharmaceutically acceptable carriers and
 30 routes of administration known to those skilled in the art. Accordingly, the present disclosure also encompasses pharmaceutical compositions comprising one or more of the DACRA peptides, DACRA conjugates or DACRA prodrug derivatives disclosed herein, in combination with a pharmaceutically acceptable carrier. The

pharmaceutical compositions may comprise the DACRA peptides, DACRA conjugates or DACRA prodrug derivatives as the sole pharmaceutically active component, or the DACRA peptides, DACRA conjugates or DACRA prodrug derivatives, can be combined with one or more additional active agents, including for example an additional anti-obesity peptide. Suitable anti-obesity peptides include those disclosed in US patents 5,691,309, 6,436,435 or US Patent application 20050176643, and including, but not limited to GLP-1, GIP (Gastric Inhibitory Polypeptide), glucagon, MP1, PYY, MC-4, NK2, Leptin. In one embodiment conjugates are prepared comprising one or more known anti-obesity peptide and the DACRA peptides, or prodrug derivatives thereof.

In accordance with some embodiments a pharmaceutical composition is provided comprising any of the DACRA peptides, DACRA conjugates or DACRA prodrug derivatives disclosed herein, preferably sterile and preferably at a purity level of at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99%, and a pharmaceutically acceptable diluent, carrier or excipient. Such compositions may contain a bioactive peptide prodrug derivative as disclosed herein, wherein the resulting active peptide is present at a concentration of at least 0.5 mg/ml, 1 mg/ml, 2 mg/ml, 3 mg/ml, 4 mg/ml, 5 mg/ml, 6 mg/ml, 7 mg/ml, 8 mg/ml, 9 mg/ml, 10 mg/ml, 11 mg/ml, 12 mg/ml, 13 mg/ml, 14 mg/ml, 15 mg/ml, 16 mg/ml, 17 mg/ml, 18 mg/ml, 19 mg/ml, 20 mg/ml, 21 mg/ml, 22 mg/ml, 23 mg/ml, 24 mg/ml, 25 mg/ml or higher.

The pharmaceutical composition can comprise any pharmaceutically acceptable ingredient, including, for example, acidifying agents, additives, adsorbents, aerosol propellants, air displacement agents, alkalizing agents, anticaking agents, anticoagulants, antimicrobial preservatives, antioxidants, antiseptics, bases, binders, buffering agents, chelating agents, coating agents, coloring agents, desiccants, detergents, diluents, disinfectants, disintegrants, dispersing agents, dissolution enhancing agents, dyes, emollients, emulsifying agents, emulsion stabilizers, fillers, film forming agents, flavor enhancers, flavoring agents, flow enhancers, gelling agents, granulating agents, humectants, lubricants, mucoadhesives, ointment bases, ointments, oleaginous vehicles, organic bases, pastille bases, pigments, plasticizers, polishing agents, preservatives, sequestering agents, skin penetrants, solubilizing agents, solvents, stabilizing agents, suppository bases, surface active agents, surfactants, suspending agents, sweetening agents, therapeutic agents, thickening

agents, tonicity agents, toxicity agents, viscosity-increasing agents, water-absorbing agents, water-miscible cosolvents, water softeners, or wetting agents.

In some embodiments, the pharmaceutical composition comprises any one or a combination of the following components: acacia, acesulfame potassium, acetyltributyl citrate, acetyltriethyl citrate, agar, albumin, alcohol, dehydrated alcohol, 5 denatured alcohol, dilute alcohol, aleuritic acid, alginic acid, aliphatic polyesters, alumina, aluminum hydroxide, aluminum stearate, amylopectin, α -amylase, ascorbic acid, ascorbyl palmitate, aspartame, bacteriostatic water for injection, bentonite, bentonite magma, benzalkonium chloride, benzethonium chloride, benzoic acid, 10 benzyl alcohol, benzyl benzoate, bronopol, butylated hydroxyanisole, butylated hydroxytoluene, butylparaben, butylparaben sodium, calcium alginate, calcium ascorbate, calcium carbonate, calcium cyclamate, dibasic anhydrous calcium phosphate, dibasic dehydrate calcium phosphate, tribasic calcium phosphate, calcium propionate, calcium silicate, calcium sorbate, calcium stearate, calcium sulfate, 15 calcium sulfate hemihydrate, canola oil, carbomer, carbon dioxide, carboxymethyl cellulose calcium, carboxymethyl cellulose sodium, β -carotene, carrageenan, castor oil, hydrogenated castor oil, cationic emulsifying wax, cellulose acetate, cellulose acetate phthalate, ethyl cellulose, microcrystalline cellulose, powdered cellulose, silicified microcrystalline cellulose, sodium carboxymethyl cellulose, cetostearyl 20 alcohol, cetrimide, cetyl alcohol, chlorhexidine, chlorobutanol, chlorocresol, cholesterol, chlorhexidine acetate, chlorhexidine gluconate, chlorhexidine hydrochloride, chlorodifluoroethane (HCFC), chlorodifluoromethane, chlorofluorocarbons (CFC)chlorophenoxyethanol, chloroxylenol, corn syrup solids, anhydrous citric acid, citric acid monohydrate, cocoa butter, coloring agents, corn oil, 25 cottonseed oil, cresol, m-cresol, o-cresol, p-cresol, croscarmellose sodium, crospovidone, cyclamic acid, cyclodextrins, dextrans, dextrin, dextrose, dextrose anhydrous, diazolidinyl urea, dibutyl phthalate, dibutyl sebacate, diethanolamine, diethyl phthalate, difluoroethane (HFC), dimethyl- β -cyclodextrin, cyclodextrin-type compounds such as Captisol®, dimethyl ether, dimethyl phthalate, dipotassium 30 edentate, disodium edentate, disodium hydrogen phosphate, docusate calcium, docusate potassium, docusate sodium, dodecyl gallate, dodecyltrimethylammonium bromide, edentate calcium disodium, edtic acid, eglumine, ethyl alcohol, ethylcellulose, ethyl gallate, ethyl laurate, ethyl maltol, ethyl oleate, ethylparaben, ethylparaben potassium, ethylparaben sodium, ethyl vanillin, fructose, fructose liquid,

fructose milled, fructose pyrogen-free, powdered fructose, fumaric acid, gelatin, glucose, liquid glucose, glyceride mixtures of saturated vegetable fatty acids, glycerin, glyceryl behenate, glyceryl monooleate, glyceryl monostearate, self-emulsifying glyceryl monostearate, glyceryl palmitostearate, glycine, glycols, glycofurol, guar

5 gum, heptafluoropropane (HFC), hexadecyltrimethylammonium bromide, high fructose syrup, human serum albumin, hydrocarbons (HC), dilute hydrochloric acid, hydrogenated vegetable oil, type II, hydroxyethyl cellulose, 2-hydroxyethyl- β -cyclodextrin, hydroxypropyl cellulose, low-substituted hydroxypropyl cellulose, 2-hydroxypropyl- β -cyclodextrin, hydroxypropyl methylcellulose, hydroxypropyl

10 methylcellulose phthalate, imidurea, indigo carmine, ion exchangers, iron oxides, isopropyl alcohol, isopropyl myristate, isopropyl palmitate, isotonic saline, kaolin, lactic acid, lactitol, lactose, lanolin, lanolin alcohols, anhydrous lanolin, lecithin, magnesium aluminum silicate, magnesium carbonate, normal magnesium carbonate, magnesium carbonate anhydrous, magnesium carbonate hydroxide, magnesium

15 hydroxide, magnesium lauryl sulfate, magnesium oxide, magnesium silicate, magnesium stearate, magnesium trisilicate, magnesium trisilicate anhydrous, malic acid, malt, maltitol, maltitol solution, maltodextrin, maltol, maltose, mannitol, medium chain triglycerides, meglumine, menthol, methylcellulose, methyl methacrylate, methyl oleate, methylparaben, methylparaben potassium,

20 methylparaben sodium, microcrystalline cellulose and carboxymethylcellulose sodium, mineral oil, light mineral oil, mineral oil and lanolin alcohols, oil, olive oil, monoethanolamine, montmorillonite, octyl gallate, oleic acid, palmitic acid, paraffin, peanut oil, petrolatum, petrolatum and lanolin alcohols, pharmaceutical glaze, phenol, liquified phenol, phenoxyethanol, phenoxypropanol, phenylethyl alcohol,

25 phenylmercuric acetate, phenylmercuric borate, phenylmercuric nitrate, polacrillin, polacrillin potassium, poloxamer, polydextrose, polyethylene glycol, polyethylene oxide, polyacrylates, polyethylene-polyoxypropylene-block polymers, polymethacrylates, polyoxyethylene alkyl ethers, polyoxyethylene castor oil derivatives, polyoxyethylene sorbitol fatty acid esters, polyoxyethylene stearates,

30 polyvinyl alcohol, polyvinyl pyrrolidone, potassium alginate, potassium benzoate, potassium bicarbonate, potassium bisulfite, potassium chloride, potassium citrate, potassium citrate anhydrous, potassium hydrogen phosphate, potassium metabisulfite, monobasic potassium phosphate, potassium propionate, potassium sorbate, povidone, propanol, propionic acid, propylene carbonate, propylene glycol, propylene glycol

alginate, propyl gallate, propylparaben, propylparaben potassium, propylparaben sodium, protamine sulfate, rapeseed oil, Ringer's solution, saccharin, saccharin ammonium, saccharin calcium, saccharin sodium, safflower oil, saponite, serum proteins, sesame oil, colloidal silica, colloidal silicon dioxide, sodium alginate,

5 sodium ascorbate, sodium benzoate, sodium bicarbonate, sodium bisulfite, sodium chloride, anhydrous sodium citrate, sodium citrate dehydrate, sodium chloride, sodium cyclamate, sodium edentate, sodium dodecyl sulfate, sodium lauryl sulfate, sodium metabisulfite, sodium phosphate, dibasic, sodium phosphate, monobasic, sodium phosphate, tribasic, anhydrous sodium propionate, sodium propionate, sodium

10 sorbate, sodium starch glycolate, sodium stearyl fumarate, sodium sulfite, sorbic acid, sorbitan esters (sorbitan fatty esters), sorbitol, sorbitol solution 70%, soybean oil, spermaceti wax, starch, corn starch, potato starch, pregelatinized starch, sterilizable maize starch, stearic acid, purified stearic acid, stearyl alcohol, sucrose, sugars, compressible sugar, confectioner's sugar, sugar spheres, invert sugar, *Sugartab*,

15 Sunset Yellow FCF, synthetic paraffin, talc, tartaric acid, tartrazine, tetrafluoroethane (HFC), theobroma oil, thimerosal, titanium dioxide, alpha tocopherol, tocopheryl acetate, alpha tocopheryl acid succinate, beta-tocopherol, delta-tocopherol, gamma-tocopherol, tragacanth, triacetin, tributyl citrate, triethanolamine, triethyl citrate, trimethyl- β -cyclodextrin, trimethyltetradecylammonium bromide, tris buffer,

20 trisodium edentate, vanillin, type I hydrogenated vegetable oil, water, soft water, hard water, carbon dioxide-free water, pyrogen-free water, water for injection, sterile water for inhalation, sterile water for injection, sterile water for irrigation, waxes, anionic emulsifying wax, carnauba wax, cationic emulsifying wax, cetyl ester wax, microcrystalline wax, nonionic emulsifying wax, suppository wax, white wax, yellow

25 wax, white petrolatum, wool fat, xanthan gum, xylitol, zein, zinc propionate, zinc salts, zinc stearate, or any excipient in the *Handbook of Pharmaceutical Excipients*, Third Edition, A. H. Kibbe (Pharmaceutical Press, London, UK, 2000), which is incorporated by reference in its entirety. *Remington's Pharmaceutical Sciences*, Sixteenth Edition, E. W. Martin (Mack Publishing Co., Easton, Pa., 1980), which is

30 incorporated by reference in its entirety, discloses various components used in formulating pharmaceutically acceptable compositions and known techniques for the preparation thereof. Except insofar as any conventional agent is incompatible with the pharmaceutical compositions, its use in pharmaceutical compositions is

contemplated. Supplementary active ingredients also can be incorporated into the compositions.

The pharmaceutical formulations disclosed herein may be designed to be short-acting, fast-releasing, long-acting, or sustained-releasing as described below.

5 The pharmaceutical formulations may also be formulated for immediate release, controlled release or for slow release. The instant compositions may further comprise, for example, micelles or liposomes, or some other encapsulated form, or may be administered in an extended release form to provide a prolonged storage and/or delivery effect. The disclosed pharmaceutical formulations may be
10 administered according to any regime including, for example, daily (1 time per day, 2 times per day, 3 times per day, 4 times per day, 5 times per day, 6 times per day), every two days, every three days, every four days, every five days, every six days, weekly, bi-weekly, every three weeks, monthly, or bi-monthly.

In some embodiments, the foregoing component(s) may be present in the
15 pharmaceutical composition at any concentration, such as, for example, at least A, wherein A is 0.0001% w/v, 0.001% w/v, 0.01% w/v, 0.1% w/v, 1% w/v, 2% w/v, 5% w/v, 10% w/v, 20% w/v, 30% w/v, 40% w/v, 50% w/v, 60% w/v, 70% w/v, 80% w/v, or 90% w/v. In some embodiments, the foregoing component(s) may be present in the pharmaceutical composition at any concentration, such as, for example, at most B,
20 wherein B is 90% w/v, 80% w/v, 70% w/v, 60% w/v, 50% w/v, 40% w/v, 30% w/v, 20% w/v, 10% w/v, 5% w/v, 2% w/v, 1% w/v, 0.1% w/v, 0.001% w/v, or 0.0001%. In other embodiments, the foregoing component(s) may be present in the pharmaceutical composition at any concentration range, such as, for example from about A to about B. In some embodiments, A is 0.0001% and B is 90%.

25 The pharmaceutical compositions may be formulated to achieve a physiologically compatible pH. In some embodiments, the pH of the pharmaceutical composition may be at least 5, at least 5.5, at least 6, at least 6.5, at least 7, at least 7.5, at least 8, at least 8.5, at least 9, at least 9.5, at least 10, or at least 10.5 up to and including pH 11, depending on the formulation and route of administration. In certain
30 embodiments, the pharmaceutical compositions may comprise buffering agents to achieve a physiological compatible pH. The buffering agents may include any compounds capable of buffering at the desired pH such as, for example, phosphate buffers (e.g. PBS), triethanolamine, Tris, bicine, TAPS, tricine, HEPES, TES, MOPS, PIPES, cacodylate, MES, and others. In certain embodiments, the strength of the

buffer is at least 0.5 mM, at least 1 mM, at least 5 mM, at least 10 mM, at least 20 mM, at least 30 mM, at least 40 mM, at least 50 mM, at least 60 mM, at least 70 mM, at least 80 mM, at least 90 mM, at least 100 mM, at least 120 mM, at least 150 mM, or at least 200 mM. In some embodiments, the strength of the buffer is no more than
5 300 mM (e.g. at most 200 mM, at most 100 mM, at most 90 mM, at most 80 mM, at most 70 mM, at most 60 mM, at most 50 mM, at most 40 mM, at most 30 mM, at most 20 mM, at most 10 mM, at most 5 mM, at most 1 mM).

In some embodiments the pharmaceutical compositions comprise aqueous solutions that are sterilized and optionally stored within various containers. In one
10 embodiment aqueous formulations of any of the novel DACRA peptides disclosed herein, or prodrug derivatives thereof, or the clacitonin selective analogs disclosed herein, or prodrugs thereof, are prepared at concentrations of about 1mg/ml to about 20mg/ml or about 5mg/ml to about 10 mg/ml. In one embodiment pharmaceutical formulations of these peptides are prepared in dimethyl sulfoxide (DMSO), including
15 about 30% to 100% DMSO, about 50% to 100% DMSO, about 70% to 100% DMSO, or about 90% to 100% DMSO, with the remainder water. In one embodiment aqueous formulations are prepared in 100% dimethyl sulfoxide. The compounds of the present invention can be used in accordance with some embodiments to prepare pre-formulated solutions ready for injection. In other embodiments the pharmaceutical
20 compositions comprise a lyophilized powder. The pharmaceutical compositions can be further packaged as part of a kit that includes a disposable device for administering the composition to a patient. The containers or kits may be labeled for storage at ambient room temperature or at refrigerated temperature.

All therapeutic methods, pharmaceutical compositions, kits and other similar
25 embodiments described herein contemplate that the prodrug compounds include all pharmaceutically acceptable salts thereof.

In some embodiments the kit is provided with a device for administering the prodrug composition to a patient. The kit may further include a variety of containers, e.g., vials, tubes, bottles, and the like. Preferably, the kits will also include
30 instructions for use. In accordance with some embodiments the administering element of the kit is an aerosol dispensing device, wherein the composition is prepackaged within the aerosol device. In another embodiment the kit comprises a syringe and a needle, and in some embodiments the prodrug composition is prepackaged within the syringe.

The present DACRA peptides, and the prodrug derivatives thereof, have use in the treatment of diabetes, metabolic syndrome and for reducing weight gain or inducing weight loss, particularly when conjugated to an incretin peptide.

Pharmaceutical compositions comprising the novel DACRA peptides and calcitonin selective peptides disclosed herein, and the prodrug derivatives thereof, and salts thereof, can be administered by any standard route including by oral or inhalation administration.

The present disclosure also encompasses multimers of the DACRA peptides disclosed herein. Two or more of the DACRA peptides can be linked together using standard linking agents and procedures known to those skilled in the art. For example, dimers can be formed between two DACRA peptides through the use of bifunctional thiol crosslinkers and bi-functional amine crosslinkers, particularly for the DACRA peptides comprising cysteine, lysine ornithine, homocysteine or acetyl phenylalanine residues.

Routes of Administration

The following discussion on routes of administration is merely provided to illustrate exemplary embodiments and should not be construed as limiting the scope in any way.

Formulations suitable for oral administration can consist of (a) liquid solutions, such as an effective amount of the DACRA peptide dissolved in diluents, such as water, saline, DMSO, or orange juice; (b) capsules, sachets, tablets, lozenges, and troches, each containing a predetermined amount of the active ingredient, as solids or granules; (c) powders; (d) suspensions in an appropriate liquid; and (e) suitable emulsions. Liquid formulations may include diluents, such as water, DMSO and alcohols, for example, ethanol, benzyl alcohol, and the polyethylene alcohols, either with or without the addition of a pharmaceutically acceptable surfactant. Capsule forms can be of the ordinary hard- or soft-shelled gelatin type containing, for example, surfactants, lubricants, and inert fillers, such as lactose, sucrose, calcium phosphate, and corn starch. Tablet forms can include one or more of lactose, sucrose, mannitol, corn starch, potato starch, alginic acid, microcrystalline cellulose, acacia, gelatin, guar gum, colloidal silicon dioxide, croscarmellose sodium, talc, magnesium stearate, calcium stearate, zinc stearate, stearic acid, and other excipients, colorants, diluents, buffering agents, disintegrating agents, moistening agents, preservatives, flavoring agents, and other pharmacologically compatible

excipients. Lozenge forms can comprise the DACRA peptide in a flavor, usually sucrose and acacia or tragacanth, as well as pastilles comprising the DACRA peptide in an inert base, such as gelatin and glycerin, or sucrose and acacia, emulsions, gels, and the like containing, in addition to, such excipients as are known in the art.

5 The DACRA peptide, alone or in combination with other suitable components, can be delivered via pulmonary administration and can be made into aerosol formulations to be administered via inhalation. These aerosol formulations can be placed into pressurized acceptable propellants, such as dichlorodifluoromethane, propane, nitrogen, and the like. They also may be formulated as pharmaceuticals for
10 non-pressured preparations, such as in a nebulizer or an atomizer. Such spray formulations also may be used to spray mucosa. In some embodiments, the DACRA peptide is formulated into a powder blend or into microparticles or nanoparticles. Suitable pulmonary formulations are known in the art. See, e.g., Qian et al., *Int J Pharm* 366: 218-220 (2009); Adjei and Garren, *Pharmaceutical Research*, 7(6): 565-
15 569 (1990); Kawashima et al., *J Controlled Release* 62(1-2): 279-287 (1999); Liu et al., *Pharm Res* 10(2): 228-232 (1993); International Patent Application Publication Nos. WO 2007/133747 and WO 2007/141411.

Formulations suitable for parenteral administration include aqueous and non-aqueous, isotonic sterile injection solutions, which can contain antioxidants, buffers,
20 bacteriostats, and solutes that render the formulation isotonic with the blood of the intended recipient, and aqueous and non-aqueous sterile suspensions that can include suspending agents, solubilizers, thickening agents, stabilizers, and preservatives. The term, "parenteral" means not through the alimentary canal but by some other route such as subcutaneous, intramuscular, intraspinal, or intravenous. The DACRA
25 peptide can be administered with a physiologically acceptable diluent in a pharmaceutical carrier, such as a sterile liquid or mixture of liquids, including water, saline, aqueous dextrose and related sugar solutions, an alcohol, such as ethanol or hexadecyl alcohol, a glycol, such as propylene glycol or polyethylene glycol, dimethylsulfoxide, glycerol, ketals such as 2,2- dimethyl-153-dioxolane-4-methanol,
30 ethers, poly(ethyleneglycol) 40, oils, fatty acids, fatty acid esters or glycerides, or acetylated fatty acid glycerides with or without the addition of a pharmaceutically acceptable surfactant, such as a soap or a detergent, suspending agent, such as pectin, carbomers, methylcellulose, hydroxypropylmethylcellulose, or carboxymethylcellulose, or emulsifying agents and other pharmaceutical adjuvants.

Oils, which can be used in parenteral formulations include petroleum, animal, vegetable, or synthetic oils. Specific examples of oils include peanut, soybean, sesame, cottonseed, corn, olive, petrolatum, and mineral. Suitable fatty acids for use in parenteral formulations include oleic acid, stearic acid, and isostearic acid. Ethyl
5 oleate and isopropyl myristate are examples of suitable fatty acid esters.

Suitable soaps for use in parenteral formulations include fatty alkali metal, ammonium, and triethanolamine salts, and suitable detergents include (a) cationic detergents such as, for example, dimethyl dialkyl ammonium halides, and alkyl pyridinium halides, (b) anionic detergents such as, for example, alkyl, aryl, and olefin
10 sulfonates, alkyl, olefin, ether, and monoglyceride sulfates, and sulfosuccinates, (c) nonionic detergents such as, for example, fatty amine oxides, fatty acid alkanolamides, and polyoxyethylenepolypropylene copolymers, (d) amphoteric detergents such as, for example, alkyl- β -aminopropionates, and 2-alkyl -imidazoline quaternary ammonium salts, and (e) mixtures thereof.

15 The parenteral formulations will typically contain from about 0.5% to about 25% by weight of the DACRA peptide in solution. Preservatives and buffers may be used. In order to minimize or eliminate irritation at the site of injection, such compositions may contain one or more nonionic surfactants having a hydrophile-lipophile balance (HLB) of from about 12 to about 17. The quantity of surfactants in
20 such formulations will typically range from about 5% to about 15% by weight. Suitable surfactants include polyethylene glycol sorbitan fatty acid esters, such as sorbitan monooleate and the high molecular weight adducts of ethylene oxide with a hydrophobic base, formed by the condensation of propylene oxide with propylene glycol. The parenteral formulations can be presented in unit-dose or multi-dose
25 sealed containers, such as ampoules and vials, and can be stored in a freeze-dried (lyophilized) condition requiring only the addition of the sterile liquid excipient, for example, water, for injections, immediately prior to use. Extemporaneous injection solutions and suspensions can be prepared from sterile powders, granules, and tablets of the kind previously described.

30 Injectable formulations are in accordance with the invention. The requirements for effective pharmaceutical carriers for injectable compositions are well-known to those of ordinary skill in the art (see, e.g., *Pharmaceutics and Pharmacy Practice*, J. B. Lippincott Company, Philadelphia, PA, Banker and

Chalmers, eds., pages 238-250 (1982), and *ASHP Handbook on Injectable Drugs*, Toissel, 4th ed., pages 622-630 (1986)).

Additionally, the DACRA peptide can be made into suppositories for rectal administration by mixing with a variety of bases, such as emulsifying bases or water-
5 soluble bases. Formulations suitable for vaginal administration can be presented as pessaries, tampons, creams, gels, pastes, foams, or spray formulas containing, in addition to the active ingredient, such carriers as are known in the art to be appropriate.

It will be appreciated by one of skill in the art that, in addition to the above-
10 described pharmaceutical compositions, the DACRA peptide can be formulated as inclusion complexes, such as cyclodextrin inclusion complexes, or liposomes.

Dose

The compositions of the present disclosures comprising a DACRA and a peptide as described herein are believed to be useful in methods of treating a disease
15 or medical condition in which amylin or calcitonin receptor agonism play a role. For purposes of the present disclosures, the amount or dose of the composition of the present disclosure administered should be sufficient to effect, e.g., a therapeutic or prophylactic response, in the subject or animal over a reasonable time frame. For example, the dose of the composition of the present disclosures should be sufficient to
20 stimulate cAMP secretion from cells as described herein or sufficient to decrease blood glucose levels, fat levels, food intake levels, or body weight of a mammal, in a period of from about 1 to 4 minutes, 1 to 4 hours or 1 to 4 weeks or longer, e.g., 5 to 20 or more weeks, from the time of administration. In certain embodiments, the time period could be even longer. The dose will be determined by the efficacy of the
25 particular composition of the present disclosure and the condition of the animal (e.g., human), as well as the body weight of the animal (e.g., human) to be treated.

Many assays for determining an administered dose are known in the art. For purposes herein, an assay, which comprises comparing the extent to which blood glucose levels or body weight are lowered upon administration of a given dose of the
30 composition of the present disclosures to a mammal among a set of mammals of which is each given a different dose of the composition, could be used to determine a starting dose to be administered to a mammal. The extent to which blood glucose levels or body weight are lowered upon administration of a certain dose can be assayed by methods known in the art.

Typically, the attending physician will decide the dosage of the composition of the present disclosure with which to treat each individual patient, taking into consideration a variety of factors, such as age, body weight, general health, diet, sex, composition of the present disclosure to be administered, route of administration, severity of the condition being treated, and clinical effect to be achieved. The dose of the composition of the present disclosure also will be determined by the existence, nature and extent of any adverse side effects that might accompany the administration of a particular peptide of the present disclosure.

Combinations

The DACRA peptides disclosed herein may be administered alone or in combination with other therapeutic agents which aim to treat or prevent any of the diseases or medical conditions described herein. For example, the peptides described herein may be co-administered with (simultaneously or sequentially) an anti-diabetic or anti-obesity agent. Anti-diabetic agents known in the art or under investigation include insulin, leptin, incretins, Peptide YY (PYY), Pancreatic Peptide (PP), fibroblast growth factor 21 (FGF21), Y2Y4 receptor agonists, NK2, sulfonylureas, such as tolbutamide (Orinase), acetohexamide (Dymelor), tolazamide (Tolinase), chlorpropamide (Diabinese), glipizide (Glucotrol), glyburide (Diabeta, Micronase, Glynase), glimepiride (Amaryl), or gliclazide (Diamicron); meglitinides, such as repaglinide (Prandin) or nateglinide (Starlix); biguanides such as metformin (Glucophage) or phenformin; thiazolidinediones such as rosiglitazone (Avandia), pioglitazone (Actos), or troglitazone (Rezulin), or other PPAR γ inhibitors; alpha glucosidase inhibitors that inhibit carbohydrate digestion, such as miglitol (Glyset), acarbose (Precose/Glucobay); exenatide (Byetta) or pramlintide; Dipeptidyl peptidase-4 (DPP-4) inhibitors such as vildagliptin or sitagliptin; SGLT (sodium-dependent glucose transporter 1) inhibitors; glucokinase activators (GKA); glucagon receptor antagonists (GRA); or FBPase (fructose 1,6-bisphosphatase) inhibitors.

Anti-obesity agents known in the art or under investigation include appetite suppressants, including phenethylamine type stimulants, phentermine (optionally with fenfluramine or dexfenfluramine), diethylpropion (Tenuate®), phendimetrazine (Prelu-2®, Bontril®), benzphetamine (Didrex®), sibutramine (Meridia®, Reductil®); rimonabant (Acomplia®), other cannabinoid receptor antagonists; oxyntomodulin; fluoxetine hydrochloride (Prozac); Qnexa (topiramate and phentermine), Excalia (bupropion and zonisamide) or Contrave (bupropion and naltrexone); or lipase

inhibitors, similar to XENICAL (Orlistat) or Cetilistat (also known as ATL-962), or GT 389-255.

The DACRA peptide in some embodiments is co-administered with an agent for treatment of non-alcoholic fatty liver disease or NASH. Agents used to treat non-alcoholic fatty liver disease include ursodeoxycholic acid (a.k.a., Actigall, URSO, and Ursodiol), Metformin (Glucophage), rosiglitazone (Avandia), Clofibrate, Gemfibrozil, Polymixin B, and Betaine.

The DACRA peptide in some embodiments is co-administered with an agent for treatment of a neurodegenerative disease, e.g., Parkinson's Disease. Anti-Parkinson's Disease agents are furthermore known in the art and include, but not limited to, levodopa, carbidopa, anticholinergics, bromocriptine, pramipexole, and ropinirole, amantadine, and rasagiline.

In view of the foregoing, the present disclosures further provide pharmaceutical compositions and kits additionally comprising one of these other therapeutic agents. The additional therapeutic agent may be administered simultaneously or sequentially with the peptide of the present disclosure. In some aspects, the peptide is administered before the additional therapeutic agent, while in other aspects, the peptide is administered after the additional therapeutic agent.

Uses

Based on the information provided for the first time herein, it is contemplated that the compositions (e.g., related pharmaceutical compositions) of the present disclosed peptides are useful for treatment of a disease or medical condition, in which e.g., the lack of activity at the amylin receptor, the calcitonin receptor, or at both receptors, is a factor in the onset and/or progression of the disease or medical condition. Accordingly, the present disclosures provide a method of treating or preventing a disease or medical condition in a patient, wherein the disease or medical condition is a disease or medical condition in which a lack of amylin receptor activation and/or calcitonin receptor activation is associated with the onset and/or progression of the disease or medical condition. The method comprises providing the patient a composition or conjugate in accordance with any of those described herein in an amount effective to treat or prevent the disease or medical condition.

In some embodiments, the disease or medical condition is metabolic syndrome. Metabolic Syndrome, also known as metabolic syndrome X, insulin resistance syndrome or Reaven's syndrome, is a disorder that affects over 50 million

Americans. Metabolic Syndrome is typically characterized by a clustering of at least three or more of the following risk factors: (1) abdominal obesity (excessive fat tissue in and around the abdomen), (2) atherogenic dyslipidemia (blood fat disorders including high triglycerides, low HDL cholesterol and high LDL cholesterol that
5 enhance the accumulation of plaque in the artery walls), (3) elevated blood pressure, (4) insulin resistance or glucose intolerance, (5) prothrombotic state (e.g., high fibrinogen or plasminogen activator inhibitor-1 in blood), and (6) pro-inflammatory state (e.g., elevated C-reactive protein in blood). Other risk factors may include aging, hormonal imbalance and genetic predisposition.

10 Metabolic Syndrome is associated with an increased risk of coronary heart disease and other disorders related to the accumulation of vascular plaque, such as stroke and peripheral vascular disease, referred to as atherosclerotic cardiovascular disease (ASCVD). Patients with Metabolic Syndrome may progress from an insulin resistant state in its early stages to full blown type II diabetes with further increasing
15 risk of ASCVD. Without intending to be bound by any particular theory, the relationship between insulin resistance, Metabolic Syndrome and vascular disease may involve one or more concurrent pathogenic mechanisms including impaired insulin-stimulated vasodilation, insulin resistance-associated reduction in NO availability due to enhanced oxidative stress, and abnormalities in adipocyte-derived
20 hormones such as adiponectin (Lteif and Mather, Can. J. Cardiol. 20 (suppl. B):66B-76B (2004)).

According to the 2001 National Cholesterol Education Program Adult Treatment Panel (ATP III), any three of the following traits in the same individual meet the criteria for Metabolic Syndrome: (a) abdominal obesity (a waist
25 circumference over 102 cm in men and over 88 cm in women); (b) serum triglycerides (150 mg/dl or above); (c) HDL cholesterol (40 mg/dl or lower in men and 50 mg/dl or lower in women); (d) blood pressure (130/85 or more); and (e) fasting blood glucose (110 mg/dl or above). According to the World Health Organization (WHO), an individual having high insulin levels (an elevated fasting blood glucose or an elevated
30 post meal glucose alone) with at least two of the following criteria meets the criteria for Metabolic Syndrome: (a) abdominal obesity (waist to hip ratio of greater than 0.9, a body mass index of at least 30 kg/m², or a waist measurement over 37 inches); (b) cholesterol panel showing a triglyceride level of at least 150 mg/dl or an HDL cholesterol lower than 35 mg/dl; (c) blood pressure of 140/90 or more, or on treatment

for high blood pressure). (Mathur, Ruchi, "Metabolic Syndrome," ed. Shiel, Jr., William C., MedicineNet.com, May 11, 2009).

For purposes herein, if an individual meets the criteria of either or both criteria set forth by the 2001 National Cholesterol Education Program Adult Treatment Panel
5 or the WHO, that individual is considered as afflicted with Metabolic Syndrome.

Without being bound to any particular theory, compositions and conjugates described herein are useful for treating Metabolic Syndrome. Accordingly, the invention provides a method of preventing or treating Metabolic Syndrome, or
10 reducing one, two, three or more risk factors thereof, in a subject, comprising providing to the subject a composition described herein in an amount effective to prevent or treat Metabolic Syndrome, or the risk factor thereof.

In some embodiments, the method treats a hyperglycemic medical condition. In certain aspects, the hyperglycemic medical condition is diabetes, diabetes mellitus type I, diabetes mellitus type II, or gestational diabetes, either insulin-dependent or
15 non-insulin-dependent. In some aspects, the method treats the hyperglycemic medical condition by reducing one or more complications of diabetes including nephropathy, retinopathy and vascular disease.

In some aspects, the disease or medical condition is obesity. In some aspects, the obesity is drug-induced obesity. In some aspects, the method treats obesity by
20 preventing or reducing weight gain or increasing weight loss in the patient. In some aspects, the method treats obesity by reducing appetite, decreasing food intake, lowering the levels of fat in the patient, or decreasing the rate of movement of food through the gastrointestinal system.

Because obesity is associated with the onset or progression of other diseases,
25 the methods of treating obesity are further useful in methods of reducing complications associated with obesity including vascular disease (coronary artery disease, stroke, peripheral vascular disease, ischemia reperfusion, etc.), hypertension, onset of diabetes type II, hyperlipidemia and musculoskeletal diseases. The present disclosures accordingly provide methods of treating or preventing these obesity-
30 associated complications.

In some embodiments, the disease or medical condition is Nonalcoholic fatty liver disease (NAFLD). NAFLD refers to a wide spectrum of liver diseases ranging from simple fatty liver (steatosis), to nonalcoholic steatohepatitis (NASH), to cirrhosis (irreversible, advanced scarring of the liver). All of the stages of NAFLD have in

common the accumulation of fat (fatty infiltration) in the liver cells (hepatocytes).

Simple fatty liver is the abnormal accumulation of a certain type of fat, triglyceride, in the liver cells with no inflammation or scarring. In NASH, the fat accumulation is associated with varying degrees of inflammation (hepatitis) and scarring (fibrosis) of the liver. The inflammatory cells can destroy the liver cells (hepatocellular necrosis). In the terms "steatohepatitis" and "steatonecrosis", steato refers to fatty infiltration, hepatitis refers to inflammation in the liver, and necrosis refers to destroyed liver cells. NASH can ultimately lead to scarring of the liver (fibrosis) and then irreversible, advanced scarring (cirrhosis). Cirrhosis that is caused by NASH is the last and most severe stage in the NAFLD spectrum. (Mendler, Michel, "Fatty Liver: Nonalcoholic Fatty Liver Disease (NAFLD) and Nonalcoholic Steatohepatitis (NASH)," ed. Schoenfield, Leslie J., MedicineNet.com, August 29, 2005).

Alcoholic Liver Disease, or Alcohol-Induced Liver Disease, encompasses three pathologically distinct liver diseases related to or caused by the excessive consumption of alcohol: fatty liver (steatosis), chronic or acute hepatitis, and cirrhosis. Alcoholic hepatitis can range from a mild hepatitis, with abnormal laboratory tests being the only indication of disease, to severe liver dysfunction with complications such as jaundice (yellow skin caused by bilirubin retention), hepatic encephalopathy (neurological dysfunction caused by liver failure), ascites (fluid accumulation in the abdomen), bleeding esophageal varices (varicose veins in the esophagus), abnormal blood clotting and coma. Histologically, alcoholic hepatitis has a characteristic appearance with ballooning degeneration of hepatocytes, inflammation with neutrophils and sometimes Mallory bodies (abnormal aggregations of cellular intermediate filament proteins). Cirrhosis is characterized anatomically by widespread nodules in the liver combined with fibrosis. (Worman, Howard J., "Alcoholic Liver Disease", Columbia University Medical Center website).

Without being bound to any particular theory, the compositions and conjugates described herein are useful for the treatment of Alcoholic Liver Disease, NAFLD, or any stage thereof, including, for example, steatosis, steatohepatitis, hepatitis, hepatic inflammation, NASH, cirrhosis, or complications thereof. Accordingly, the present disclosures provides a method of preventing or treating Alcoholic Liver Disease, NAFLD, or any stage thereof, in a subject comprising providing to a subject a composition described herein in an amount effective to prevent or treat Alcoholic Liver Disease, NAFLD, or the stage thereof. Such

treatment methods include reduction in one, two, three or more of the following: liver fat content, incidence or progression of cirrhosis, incidence of hepatocellular carcinoma, signs of inflammation, e.g., abnormal hepatic enzyme levels (e.g., aspartate aminotransferase AST and/or alanine aminotransferase ALT, or LDH),
5 elevated serum ferritin, elevated serum bilirubin, and/or signs of fibrosis, e.g., elevated TGF-beta levels. In certain embodiments, the compositions are used to treat patients who have progressed beyond simple fatty liver (steatosis) and exhibit signs of inflammation or hepatitis. Such methods may result, for example, in reduction of AST and/or ALT levels.

10 The present disclosures also provides uses of the compositions described herein in treating neurodegenerative diseases, including but not limited to Alzheimer's disease, Parkinson's disease, Multiple Sclerosis, Amyotrophic Lateral Sclerosis, other demyelination related disorders, senile dementia, subcortical dementia, arteriosclerotic dementia, AIDS-associated dementia, or other dementias, a central
15 nervous system cancer, traumatic brain injury, spinal cord injury, stroke or cerebral ischemia, cerebral vasculitis, epilepsy, Huntington's disease, Tourette's syndrome, Guillain Barre syndrome, Wilson disease, Pick's disease, neuroinflammatory disorders, encephalitis, encephalomyelitis or meningitis of viral, fungal or bacterial origin, or other central nervous system infections, prion diseases, cerebellar ataxias,
20 cerebellar degeneration, spinocerebellar degeneration syndromes, Friedreichs ataxia, ataxia telangiectasia, spinal dysmyotrophy, progressive supranuclear palsy, dystonia, muscle spasticity, tremor, retinitis pigmentosa, striatonigral degeneration, mitochondrial encephalo-myopathies, neuronal ceroid lipofuscinosis, hepatic encephalopathies, renal encephalopathies, metabolic encephalopathies, toxin-induced
25 encephalopathies, and radiation-induced brain damage.

In some embodiments, the compositions are used in conjunction with parenteral administration of nutrients to non-diabetic patients in a hospital setting, e.g., to patients receiving parenteral nutrition or total parenteral nutrition. Nonlimiting examples include surgery patients, patients in comas, patients with
30 digestive tract illness, or a nonfunctional gastrointestinal tract (e.g. due to surgical removal, blockage or impaired absorptive capacity, Crohn's disease, ulcerative colitis, gastrointestinal tract obstruction, gastrointestinal tract fistula, acute pancreatitis, ischemic bowel, major gastrointestinal surgery, certain congenital gastrointestinal tract anomalies, prolonged diarrhea, or short bowel syndrome due to surgery, patients

in shock, and patients undergoing healing processes often receive parenteral administration of carbohydrates along with various combinations of lipids, electrolytes, minerals, vitamins and amino acids. The compositions comprising the DACRA and as disclosed herein, and the parenteral nutrition composition can be administered at the same time, at different times, before, or after each other, provided that the composition is exerting the desired biological effect at the time that the parenteral nutrition composition is being digested. For example, the parenteral nutrition may be administered, 1, 2 or 3 times per day, while the composition is administered once every other day, three times a week, two times a week, once a week, once every 2 weeks, once every 3 weeks, or once a month.

Kits

The present disclosures further provide kits comprising a DACRA peptide. Accordingly, in some embodiments, the kit comprises a DACRA which exhibits at least 0.1% activity of native amylin peptide at the amylin receptor, at least 0.1% activity of native calcitonin peptide at the calcitonin receptor.

In some aspects, the DACRA is packaged in containers, e.g., vials, tubes, bottles, single or multi-chambered pre-filled syringes, cartridges, infusion pumps (external or implantable), jet injectors, pre-filled pen devices and the like. In some embodiments, the DACRA is provided in the kit as a lyophilized form or in an aqueous solution.

The kits in some embodiments comprise instructions for use. In one embodiment the kit is provided with a device for administering the composition to a patient, e.g., syringe needle, pen device, jet injector or another needle-free injector. In accordance with one embodiment the administering element of the kit is an aerosol dispensing device, wherein the composition is prepackaged within the aerosol device. In another embodiment the kit comprises a syringe and a needle, and in one embodiment the sterile composition is prepackaged within the syringe.

In some embodiments, the kit comprises a pharmaceutically acceptable carrier, such as any of those described herein.

The following examples are given merely to illustrate the present invention and not in any way to limit its scope.

Exemplary Embodiments

In accordance with embodiment 1 a DACRA peptide (having amylin/calcitonin co-agonist activity is provided wherein the peptide comprises the sequence of

5 R₄₀-X₁CNTX₅TCAX₉QRLAEFLRH₁₉SNNFGX₂₅IL-W₁-X₂₈X₂₉TNVGSNTZ-R₂₀ (SEQ ID NO: 1),

R₄₀-X₁CNTX₅TCATQRLAEFLRHSS-W₁-X₂₈X₂₉TNVGSNTZ-R₂₀ (SEQ ID NO: 6), or a peptide that differs from SEQ ID NO: 1 or SEQ ID NO: 6 by 1, 2, 3, 4 or 5 amino acid substitutions, differs from SEQ ID NO: 1 or SEQ ID NO: 6 by 1 or 2
10 amino acid substitutions, or differs from SEQ ID NO: 1 or SEQ ID NO: 6 by 1 amino acid substitution, optionally wherein the substitutions are conservative amino acid substitutions, wherein

X₁ is Lys, , Orn, beta-Orn or beta-Lys, or Lys, Orn, beta-Orn or beta-Lys in the D- stereoisomer configuration, optionally wherein X₁ is Lys or beta-Lys;

15 X₅ is Ala or Ser;

X₉ is Thr, isoacyl-Thr, N-acetyl-Thr, or isoacyl-Thr acylated at the alpha amine with a self-cleaving dipeptide element (A-B), wherein A is an amino acid and B is an N-alkylated amino acid;

W₁ is absent or is a spacer linking the two adjacent amino acids on
20 either side of W₁, further wherein W₁ is a spacer comprising the structure NH₂(CH₂CH₂O)_m(CH₂)_nCOOH, wherein m is an integer selected from the range of 1-20 and n is 1 or 2, optionally wherein m is 2, 4, 6, 8 or 10 and n is 1 or 2, optionally wherein m is 8 and n is 2;

X₁₉ is Ser, N-acetyl-Ser or isoacyl-Ser acylated at the alpha amine with
25 a self-cleaving dipeptide element (A-B), wherein A is an amino acid and B is an N-alkylated amino acid;

X₂₅ is Ala, Pro or hydroxyproline;

X₂₈ is selected from the group consisting of Ser, Orn, Lys, hydroxyproline, N-alpha acyl-ornithine, N-alpha acetyl-Lys, N-acetyl-hydroxyproline
30 and isoacyl-hydroxyproline acylated at the alpha amine with a self-cleaving dipeptide element (A-B), wherein A is an amino acid and B is an N-alkylated amino acid;

X₂₉ is selected from the group consisting of Ser, Orn, Lys, hydroxyproline, N-alpha acyl-ornithine, N-alpha acetyl-Lys, N-acetyl-

hydroxyproline, isoacyl-Ser acylated at the alpha amine with a self-cleaving dipeptide element (A-B), wherein A is an amino acid and B is an N-alkylated amino acid, and isoacyl-hydroxyproline acylated at the alpha amine with a self-cleaving dipeptide element (A-B), wherein A is an amino acid and B is an N-alkylated amino acid;

5 Z is pipecolic acid, Azetidine-2-carboxylic acid, hydroxyproline or N-acyl-hydroxyproline, optionally Z is hydroxyproline or N-acyl-hydroxyproline; and

R₄₀ is absent, or is a C16-C30 fatty acid, a C16-C30 phosphonic fatty acid, a C16-C30 diacid or a C16-C30 alkyl covalently linked to the alpha amine, the beta amine, or the side chain amine of the amino acid at position 1 (X₁), optionally via
 10 a spacer; wherein said spacer is selected from the group consisting of a gamma glutamic acid, a gamma glutamic acid-gamma glutamic acid dipeptide, and a (gamma glutamic acid)_z-[COCH₂(OCH₂CH₂)_k-NH]_q-(gamma glutamic acid)_p, wherein z is 0 or 1, k is an integer selected from the range of 2-4 and q and p are independently an integer selected from the range of 0-4, with the proviso that one of z, p or q is other
 15 than 0, optionally wherein R₄₀ is γ ECOC₁₈H₃₆CO₂H, or γ ECOC₁₈H₃₆PO₃H₂; and

R₂₀ is CONH₂ or COOH, with the optional proviso that an isoacyl amino acid is present at only one or two of positions 9, 19 and 28 relative to SEQ ID NO: 1.

In accordance with embodiment 2, a peptide of embodiment 1 is provided having amylin/calcitonin co-agonist activity, wherein R₂₀ is CONH₂.

20 In accordance with embodiment 3, a peptide of embodiment 1 or 2 is provided wherein X₂₈ is selected from the group consisting of Orn, Lys, hydroxyproline, N-alpha acyl-ornithine, N-alpha acetyl-Lys or N-acetyl-hydroxyproline.

In accordance with embodiment 4, a peptide of any one of embodiments 1-3 is provided wherein X₁ is Lys, dLys, Orn, beta-Orn or beta-Lys acylated with a C16-
 25 C30 fatty acid, a C16-C30 phosphonic fatty acid, or a C16-C30 diacid (i.e., R₄₀ is present) either at the alpha amine, the beta amine or the side chain amine of the amino acid at position 1 optionally via a spacer selected from the group consisting of a gamma glutamic acid, a gamma glutamic acid-gamma glutamic acid dipeptide.

In accordance with embodiment 5, a peptide of any one of embodiments 1-4 is
 30 provided wherein X₁ is beta-Lys, acylated at the beta amine with a C16-C30 diacid, or a C16-C30 phosphonic fatty acid optionally via a spacer selected from the group consisting of a gamma glutamic acid, a gamma glutamic acid-gamma glutamic acid dipeptide.

In accordance with embodiment 6, a peptide of any one of embodiments 1-5 is provided wherein R_{40} - X_1 is beta-Lys, acylated at the beta amine with γ E-COC₁₈H₃₆CO₂H or γ E-COC₁₈H₃₆PO₃H₂.

5 In accordance with embodiment 7, a peptide of any one of embodiments 1-5 is provided wherein

R_{40} is absent; and

X_1 is Lys, dLys, Orn, beta-Orn or beta-Lys covalently linked via a spacer to a straight chain or branched polyethylene glycol chain having a molecular weight ranging from about 20k to about 40k, wherein said spacer
10 comprises

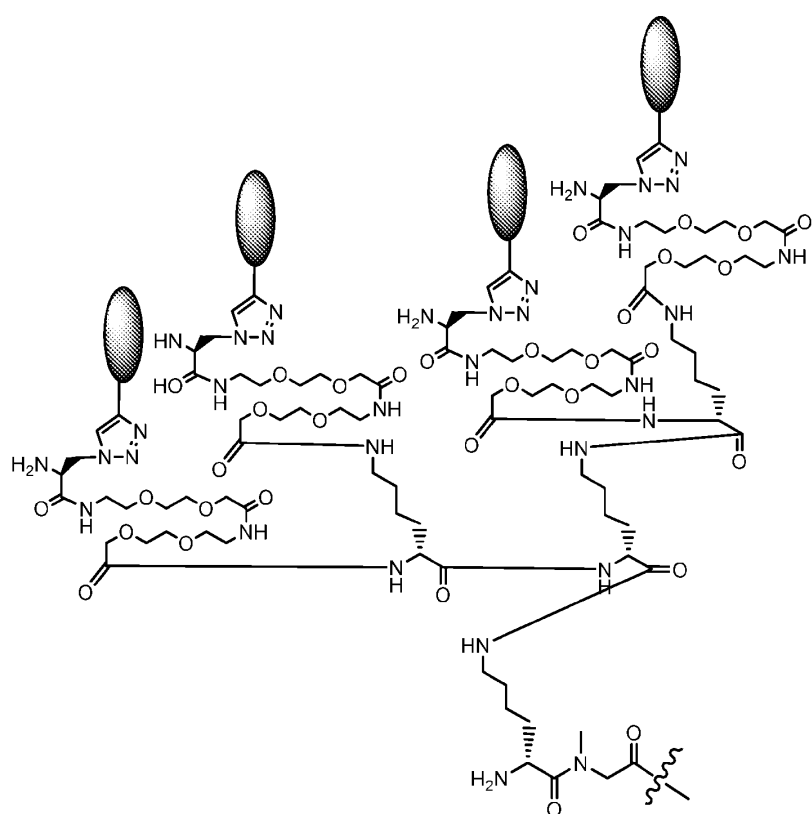
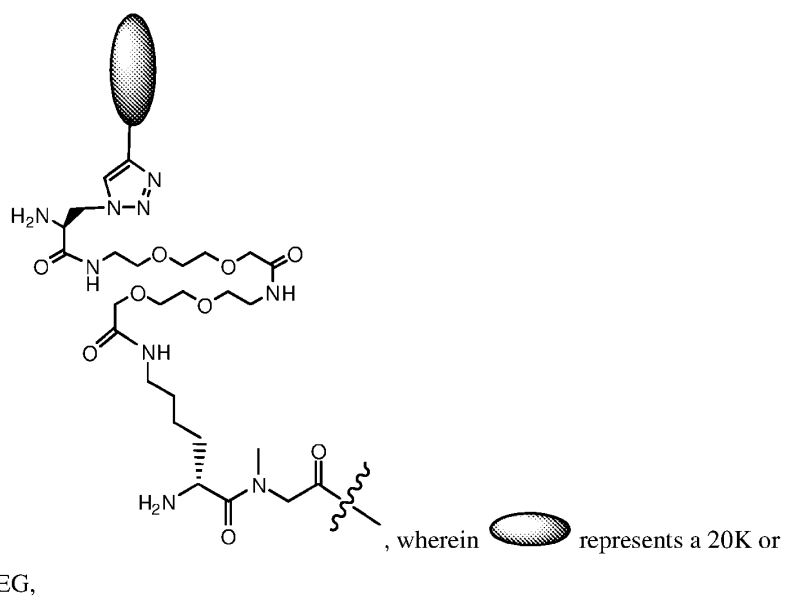
i) $-\text{[COCH}_2(\text{OCH}_2\text{CH}_2)_k\text{-NH]}_q\text{-(alanine-triazole)}$, wherein k is 2, and q is 1 or 2, optionally wherein k is 2, or

ii) $-\text{[COCH}_2(\text{OCH}_2\text{CH}_2)_k\text{-NH]}_q\text{-(cysteine-S-S)}$, wherein k is 2, and q is 1 or 2, optionally wherein k is 2.

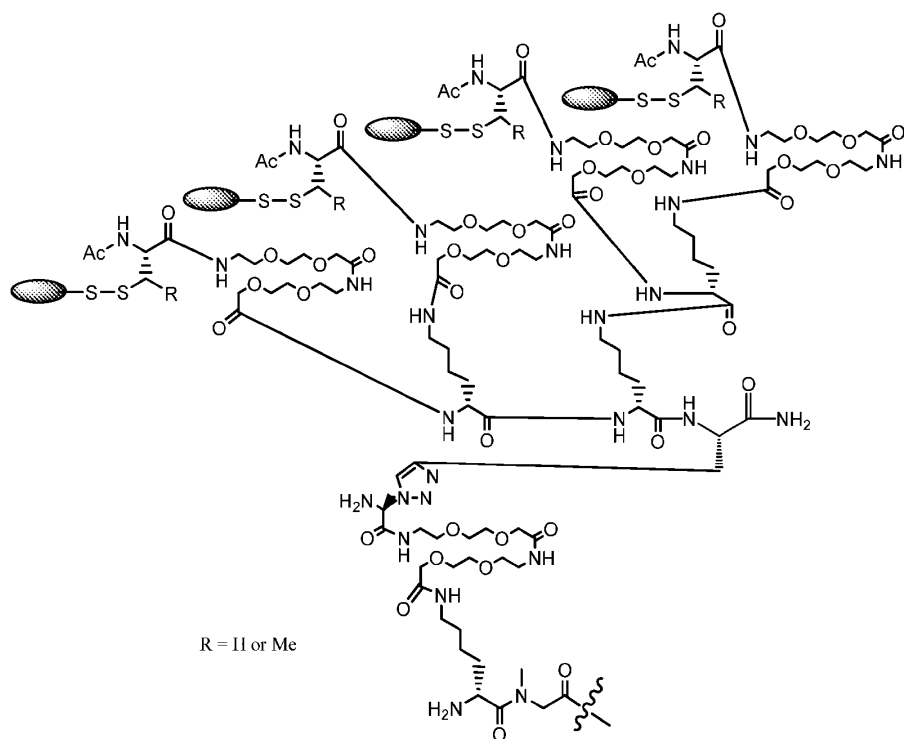
15 In accordance with embodiment 8, a peptide of embodiment 7 is provided wherein X_1 is Lys or beta-Lys that is covalently linked to a branched polyethylene chain that comprises four branches of 5K each, or four branches of 10K where each branch is linked via a spacer comprising

$-\text{[COCH}_2(\text{OCH}_2\text{CH}_2)_k\text{-NH]}_q\text{-(cysteine-S-S)}$, wherein k is 2 or 4, and q is 1 or
20 2, optionally wherein k is 2, optionally X_1 is beta-Lys and the polyethylene chain is linked to the beta amine of the beta-Lys.

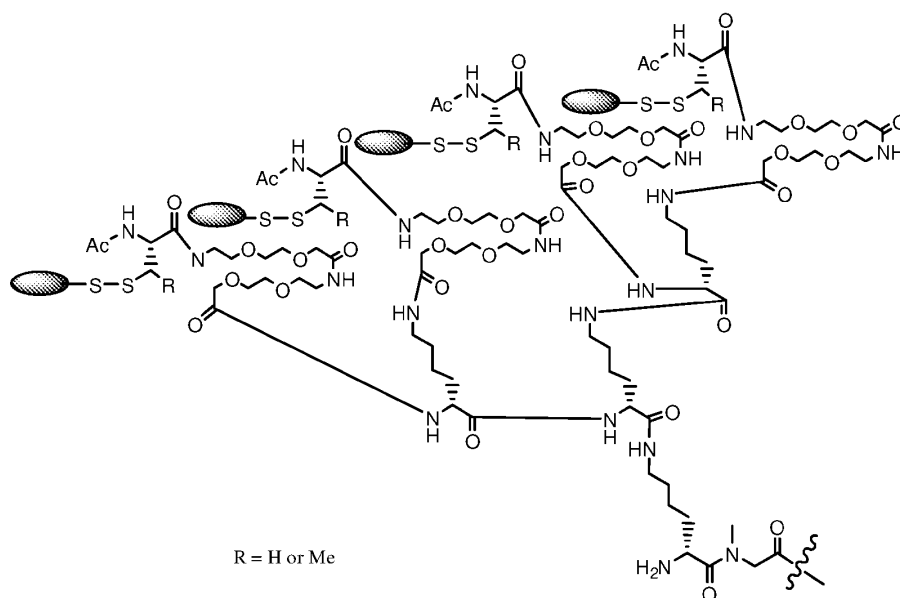
In accordance with embodiment 9, a peptide of embodiment 7 or 8 is provided wherein X_1 comprises a structure selected from:



wherein  represents a 5K or 10K PEG,



, wherein  represents a 5K or 10K PEG and Ac = acyl group, or



wherein  represents a 5K or 10K PEG and Ac = acyl group.

5 In accordance with embodiment 10, a peptide of any one of embodiments 1-9 is provided wherein W_1 is $NH_2(CH_2CH_2O)_mCH_2CH_2COOH$, wherein m is 6, 8 or 10, optionally wherein m is 8.

In accordance with embodiment 11, a peptide of any one of embodiments 1-10 is provided wherein X_{25} is Ala.

10 In accordance with embodiment 12 a peptide of any one of embodiments 1-11 is provided wherein Z is 4-hydroxyproline.

In accordance with embodiment 13 a peptide of any one of embodiments 1-12 is provided wherein X_5 is Ala.

15 In accordance with embodiment 14 a peptide of any one of embodiments 1-13 is provided wherein X_9 is Thr.

In accordance with embodiment 15 a peptide of any one of embodiments 1-14 is provided wherein X_{19} is Ser.

In accordance with embodiment 16 a peptide of any one of embodiments 1-15 is provided wherein X_{29} is Ser.

20

In accordance with embodiment 17 a peptide of any one of embodiments 1-16 is provided wherein X_{28} is hydroxyproline.

In accordance with embodiment 18 a peptide of any one of embodiments 1-16 is provided wherein X_{28} is Lys or Orn.

5 In accordance with embodiment 19 a conjugate derivative of the DACRA peptide of any one of embodiments 1-18 is provided wherein

an incretin peptide is linked to the free amine of the X_{28} amino acid of said DACRA peptide, optionally via a conjugate spacer, optionally wherein

10 i) if the amino acid at (X_{28}) is linked to W_1 via the alpha amine of said amino acid, then the incretin peptide is linked to the side chain amine of the amino acid at X_{28} ;

ii) if the amino acid at X_{28} is linked to W_1 via the side chain amine of said amino acid, then the incretin peptide is linked to the alpha amine of the amino acid at X_{28} , wherein said conjugate spacer comprises the structure: -

15 $[\text{COCH}_2(\text{OCH}_2\text{CH}_2)_k\text{NH}]_q\text{-(gamma glutamic acid)}_p\text{-}$; wherein k is 2, p is 1 or 2 and q is an integer selected from 1, 2 or 4, optionally wherein k is 2, p is 1 and q is 2.

In accordance with embodiment 20, a DACRA conjugate of embodiment 19 is provided wherein X_{28} is Orn or Lys.

20 In accordance with embodiment 21, a DACRA conjugate of embodiment 19 or 20 is provided wherein X_{28} is Lys.

In accordance with embodiment 22, a DACRA conjugate of any one of embodiments 19-21 is provided wherein X_{28} is Lys, wherein the alpha amine of said Lys is linked to W_1 and the side chain amine is linked to the carboxy terminus of said incretin peptide via a conjugate spacer comprising the structure: -

25 $[\text{COCH}_2(\text{OCH}_2\text{CH}_2)_k\text{NH}]_q\text{-(gamma glutamic acid)}_p\text{-}$; wherein k is 2, p is 1 or 2 and q is an integer selected from 1, 2 or 4, optionally wherein k is 2, p is 1 and q is 2.

30 In accordance with embodiment 23, a DACRA conjugate of any one of embodiments 19-21 is provided wherein said incretin peptide exhibits agonist activity at any one of the glucagon, GIP and GLP-1 receptors, or co-agonist activity at the GIP and GLP-1 receptors, or exhibits tri-agonist activity at the glucagon, GIP and GLP-1 receptors.

In accordance with embodiment 24 the DACRA conjugate of embodiment 22 or 23 is provided wherein the incretin peptide comprises the sequence of

YX₂X₃GTX₆X₇SDYSIX₁₃LX₁₅KIAQX₂₀AFVQWLIAGGPSSGAPPPS-
R₂₀ (SEQ ID NO: 14) or

YX₂X₃GTFTSDYSIX₁₃LX₁₅KX₁₇AQX₄₀AFVQWLLEGGPSSGAPPPS-
R₂₀ (SEQ ID NO: 15)

- 5 wherein
- X₂ is Aib;
- X₃ is Glu or Gln;
- X₆ is alpha methylated Phe or Phe;
- X₇ is isoacyl-Thr, Thr, or isoacyl-Thr acylated at the alpha amine with
- 10 a self-cleaving dipeptide disclosed herein dipeptide element (A-B), wherein A is
 an amino acid and B is an N-alkylated amino acid;
- X₁₃ is Aib or alpha methylated Leu;
- X₁₅ is Glu or Asp;
- X₄₀ is Aib;
- 15 R₂₀ is CONH₂; and
- X₁₇ and X₂₀ are independently an amino acid comprising a (C1-C4
alkyl)NH₂ side chain that has been acylated with a C16-C20 acyl group, a C16-C20
phosphonic fatty acid, or a C16-C20 alkyl group, optionally via a conjugate spacer;
wherein the conjugate spacer comprises the structure: -[COCH₂(OCH₂CH₂)_kNH]_q-
20 (gamma glutamic acid)_p-; wherein k is 2, p is 1 or 2 and q is an integer selected from
1, 2 or 4, optionally wherein k is 2, p is 1 and q is 2. I

In accordance with embodiment 25, a DACRA conjugate of embodiment 24 is
provided wherein the incretin peptide comprises the sequence of SEQ ID NO: 14
wherein X₂ is Aib, X₃ is Glu, X₆ is alpha methylated Phe, X₇ is said acylated isoacyl-
25 Thr or Thr; X₁₃ is Aib, X₁₅ is Glu, X₂₀ is Lys acylated via its side chain with
(COCH₂(OCH₂CH₂)₂NH)₂-γE-COC₁₈H₃₆CO₂H, or Lys acylated via its side chain
with (COCH₂(OCH₂CH₂)₂NH)₂-γE-COC₁₈H₃₆PO₃H₂ and R₂₀ is CONH₂.

In accordance with embodiment 26, a DACRA conjugate of embodiment 24 is
provided wherein the incretin peptide comprises the sequence of SEQ ID NO: 15
30 wherein X₂ is Aib, X₃ is Gln, X₁₃ is alpha methylated Leu, and X₁₅ is Asp; X₁₇ is Lys
acylated via its side chain with (COCH₂(OCH₂CH₂)₂NH)₂-γE-COC₁₈H₃₆CO₂H or Lys
acylated via its side chain with (COCH₂(OCH₂CH₂)₂NH)₂-γE-COC₁₈H₃₆PO₃H₂, X₄₀ is
Aib and R₂₀ is CONH₂.

In accordance with embodiment 27, a DACRA conjugate of any one of embodiments 19-23 is provided wherein said incretin is selected from Semaglutide (SEQ ID NO: 10), tirzepatide (SEQ ID NO: 11), retatrutide (SEQ ID NO: 12) or known analog thereof.

- 5 In accordance with embodiment 28, DACRA conjugate of any one of embodiments 19-27 is provided comprising the structure of

$R_{40}-X_1\text{CNTATCATQRLAEFLRHSSNNFGAIL}-W_1-X_{28}\text{STNVGSNTZ-CONH}_2$ (SEQ ID NO: 4),

- 10 $R_{40}-X_1\text{CNTATCATQRLAEFLRHSS}-W_1-X_{28}\text{STNVGSNTZ-CONH}_2$ (SEQ ID NO: 8), or a peptide that differs from SEQ ID NO: 4 or SEQ ID NO: 8 by 1, 2, 3, 4 or 5 amino acid substitutions, optionally wherein the substitutions are conservative amino acid substitutions wherein

X_1 is Lys, Orn, beta-Orn or beta-Lys;

- 15 X_{28} is Lys or Orn linked to W_1 via the alpha amine or the side chain amine of the Lys or Orn;

Z is hydroxyproline, optionally 4-hydroxyproline;

- 20 W_1 is a bifunctional linker comprising the structure $\text{NH}_2(\text{CH}_2\text{CH}_2\text{O})_m(\text{CH}_2)_n\text{COOH}$, wherein m is an integer selected from the range of 1-20 and n is 1 or 2, optionally wherein m is 2, 4, 6, 8 or 10 and n is 1 or 2, optionally wherein m is 8 and n is 2; and

- 25 R_{40} is absent, or a C16-C30 fatty acid, a C16-C30 phosphonic fatty acid, a C16-C30 diacid group or a C16-C30 alkyl group covalently linked to the N-terminal alpha amine, the beta amine, or the side chain amine of the amino acid at position 1 (X_1), optionally via a first spacer; wherein said first spacer is selected from the group consisting of a gamma glutamic acid, gamma glutamic acid-gamma glutamic acid dipeptide, $[\text{NH}-(\text{CH}_2\text{CH}_2\text{O})_m-\text{COCH}_2-]_n$ and a (gamma glutamic acid) $_z$ - $[\text{COCH}_2(\text{OCH}_2\text{CH}_2)_k-\text{NH}]_q$ -(gamma glutamic acid) $_p$, wherein m is an integer selected from the range of 2-4, n is an integer selected from the range of 1-3, z is 0 or 1, k is an integer selected from the range of 2-4 and q and p are independently an integer selected from the range of 0-4, optionally wherein R_{40} is $\gamma\text{E-COC}_{18}\text{H}_{36}\text{CO}_2\text{H}$; and

an incretin peptide linked to the free amine of the amino acid at position 28 (i.e., if the amino acid at position 28 (X_{28}) is linked to W_1 via its alpha amine, then the incretin is linked to the side chain amine of the amino acid at position 28 (X_{28}) and

vice versa), optionally via a conjugate spacer, wherein said conjugate spacer is a bifunctional linker comprising the structure $\text{NH}_2(\text{CH}_2\text{CH}_2\text{O})_m(\text{CH}_2)_n\text{COOH}$, wherein m is an integer selected from the range of 1-20 and n is 1 or 2, optionally wherein m is 2, 4, 6, 8 or 10 and n is 1 or 2, optionally wherein m is 8 and n is 2.

5 In accordance with embodiment 29, the DACRA conjugate of any one of embodiments 19-28 is provided comprising the structure of

$\text{R}_{40}\text{-X}_1\text{CNTATCATQRLAEFLRHSSNNFGAIL-W}_1\text{-X}_{28}\text{STNVGSNTZ-CONH}_2$ (SEQ ID NO: 4), or

10 $\text{R}_{40}\text{-X}_1\text{CNTATCATQRLAEFLRHSS-W}_1\text{-X}_{28}\text{STNVGSNTZ-CONH}_2$ (SEQ ID NO: 8).

In accordance with embodiment 30, the DACRA conjugate of any one of embodiments 19-29 is provided comprising the structure of

$\text{R}_{40}\text{-X}_1\text{CNTATCATQRLAEFLRHSS-W}_1\text{-X}_{28}\text{STNVGSNTZ-CONH}_2$ (SEQ ID NO: 8).

15 In accordance with embodiment 31, the DACRA conjugate of any one of embodiments 19-30 is provided wherein

X_1 is beta-Lys;

X_{28} is Orn or Lys linked to W_1 via the alpha amine or the side chain amine of the Orn or Lys;

20 Z is hydroxyproline;

W_1 comprises bifunctional linker comprising the structure $\text{NH}_2(\text{CH}_2\text{CH}_2\text{O})_m(\text{CH}_2)_n\text{COOH}$, wherein m is an integer selected from the range of 1-20 and n is 1 or 2, optionally wherein m is 2, 4, 6, 8 or 10 and n is 1 or 2, optionally wherein m is 8 and n is 2; and

25 R_{40} is absent or a C16-C20 phosphonic fatty acid, or a C16-C20 diacid group covalently linked to the beta amine of beta-Lys, optionally via a first spacer; wherein said first spacer is selected from the group consisting of a gamma glutamic acid, and gamma glutamic acid-gamma glutamic acid dipeptide, optionally wherein the first spacer is gamma glutamic acid.

30 In accordance with embodiment 32, the DACRA conjugate of any one of embodiments 19-31 is provided wherein

X_{28} is Lys linked to W_1 via the alpha amine or the side chain amine of Lys.

In accordance with embodiment 33, the DACRA conjugate of any one of embodiments 1-32 is provided wherein

R₄₀ is R₄₀ is γ E-COC₁₈H₃₆CO₂H or γ E-COC₁₈H₃₆PO₃H₂ linked to the N-terminal alpha amine or beta amine of the amino acid at position 1 of said peptide.

In accordance with embodiment 34, the DACRA conjugate of any one of embodiments 19-33 is provided wherein X₂₈ is Lys, wherein the alpha amine of X₂₈ is
5 linked to W₁ and the side chain amine of said Lys is linked to the carboxy terminus of said incretin via a conjugate spacer.

In accordance with embodiment 35, the DACRA conjugate of any one of embodiments 19-34 is provided wherein said incretin is selected from Semaglutide (SEQ ID NO: 10), tirzepatide (SEQ ID NO: 11), retatrutide (SEQ ID NO: 12) or
10 known analogs thereof.

In accordance with embodiment 36, the DACRA conjugate of any one of embodiments 19-35 is provided wherein W₁ and the conjugate spacer are independently selected from NH₂(CH₂CH₂O)_mCH₂CH₂COOH, wherein m is an integer selected from the range of 1-20, optionally wherein m is 2, 4, 6, 8 or 10,
15 optionally wherein m is 8.

In accordance with embodiment 37, the DACRA conjugate of any one of embodiments 19-36 is provided wherein W₁ and the conjugate spacer are both NH₂(CH₂CH₂O)₈CH₂CH₂COOH.

In accordance with embodiment 38, the DACRA conjugate of any one of
20 embodiments 19-37 is provided, wherein the conjugate spacer comprises the structure NH₂(CH₂CH₂O)_m(CH₂)_nCOOH, wherein m is an integer selected from the range of 1-20 and n is 1 or 2, optionally wherein m is 2, 4, 6, 8 or 10 and n is 1 or 2, optionally wherein m is 8 and n is 2.

In accordance with embodiment 39 a DACRA conjugate is provided
25 comprising the structure of

R₄₀-X₁CNTATCATQRLAEFLRHSSNNFGAIL-W₁-X₂₈STNVGSNTZ-CONH₂ (SEQ ID NO: 4), or a peptide that differs from SEQ ID NO: 4 by 1, 2, 3, 4 or
5 amino acid substitutions, optionally wherein the substitutions are conservative amino acid substitutions wherein

30 X₁ is beta-Lys;

X₂₈ is Orn or Lys linked to W₁ via the alpha amine or the side chain amine of Orn or Lys;

Z is hydroxyproline;

W_1 is bifunctional linker comprising the structure

$NH_2(CH_2CH_2O)_m(CH_2)_nCOOH$, wherein m is an integer selected from the range of 1-20 and n is 1 or 2, optionally wherein m is 2, 4, 6, 8 or 10 and n is 1 or 2, optionally wherein m is 8 and n is 2; and

5 R_{40} is a C16-C30 fatty acid, a C16-C20 phosphonic fatty acid, a C16-C30 diacid group or a C16-C30 alkyl group covalently linked to the beta amine, or the side chain amine of beta-Lys, optionally via a first spacer; wherein said first spacer is selected from the group consisting of a gamma glutamic acid, and gamma glutamic acid-gamma glutamic acid dipeptide, or

10 R_{40} is absent; and

X_1 is Lys, dLys, Orn, beta-Orn or beta-Lys covalently linked via a second spacer to a straight chain or branched polyethylene glycol chain having a molecular weight ranging from about 20k to about 40k, wherein said second spacer comprises

15 i) $-[COCH_2(OCH_2CH_2)_k-NH]_q-$ (alanine-triazole), wherein k is 2, and q is 1 or 2, optionally wherein k is 2, or

ii) $-[COCH_2(OCH_2CH_2)_k-NH]_q-$ (cysteine-S-S), wherein k is 2, and q is 1 or 2, optionally wherein k is 2; and

an incretin peptide linked to

20 i) the alpha amine of the Orn or Lys at X_{28} , optionally via a conjugate spacer, wherein the side chain amine of the Orn or Lys at X_{28} is covalently linked to W_1 ; or

ii) the side chain amine of the Orn or Lys at X_{28} , optionally via a conjugate spacer, wherein the alpha amine of the Orn or Lys at X_{28} is covalently linked to W_1 , wherein the conjugate spacer comprises $NH_2(CH_2CH_2O)_2CH_2COOH$ or

25 $[NH_2(CH_2CH_2O)_mCH_2CH_2COOH]$, wherein m is an integer selected from the range of 1-20, optionally m is 2, 4, 6, 8 or 10, optionally m is 8.

In accordance with embodiment 40, the DACRA conjugate of any one of embodiments 1-39 is provided wherein W_1 and the conjugate spacer comprises the structure $[NH_2(CH_2CH_2O)_m(CH_2)_nCOOH]$, wherein m is an integer selected from the range of 1-20 and n is 1 or 2, optionally m is 2, 4, 6, 8 or 10 and n is 1 or 2, optionally m is 8 and n is 1 or 2.

In accordance with embodiment 41, the DACRA conjugate of embodiment 40 is provided wherein m is 8 and n is 2.

In accordance with embodiment 42, the DACRA conjugate of any one of embodiments 19-41 is provided wherein the incretin peptide is selected from the group consisting of Semaglutide (SEQ ID NO: 10), tirzepatide (SEQ ID NO: 11), retatrutide (SEQ ID NO: 12) and analogs thereof, wherein said analogs retain agonist activity at the GLP-1 receptor, and optionally at both the GIP and the GLP-1 receptors.

In accordance with embodiment 43, an amylin analog of any one of embodiments 1-18 is provided wherein the amylin analog has the sequence of SEQ ID NO: 1,

10 W_1 is absent;
 X_{28} is hydroxyproline; and
 X_{29} is Ser.

In accordance with embodiment 44, an amylin analog of any one of embodiments 1-18 is provided wherein the amylin analog has the sequence of SEQ ID NO: 1,

15 W_1 is absent;
 X_{28} is Ser; and
 X_{29} is hydroxyproline.

In accordance with embodiment 45, an amylin analog of any one of embodiments 1-18 is provided wherein the amylin analog has the sequence of SEQ ID NO: 6,

W_1 is absent; and
 X_{28} and X_{29} are each hydroxyproline, optionally both 3-hydroxyproline.

In accordance with embodiment 46, an amylin analog of any one of embodiments 1-18 and 43-45 is provided wherein W_1 is absent; and X_5 is Ala.

In accordance with embodiment 47, an amylin analog of any one of embodiments 1-18 and 43-46 is provided wherein W_1 is absent; and

X_{28} is hydroxyproline, N-alpha acyl-ornithine, N-alpha acyl-Lys or N-acyl-hydroxyproline;

30 In accordance with embodiment 48, an amylin analog of any one of embodiments 1-18 and 43-47 is provided wherein W_1 is absent; and
 X_{29} is Ser or hydroxyproline.

In accordance with embodiment 49, an amylin analog of any one of embodiments 1-18 and 43-48 is provided wherein W_1 is absent; and

X₂₉ is hydroxyproline

In accordance with embodiment 50, an amylin analog of any one of embodiments 1-49 is provided wherein X₂₈ is Orn.

In accordance with embodiment 51, an amylin analog is provided having amylin/calcitonin co-agonist activity wherein the peptide comprises the sequence of

R₄₀-X₁CNTX₅TCATQRLAEFLRHSSNNFGX₂₅ILX₂₈X₂₉TNVGSNTZ-R₂₀
(SEQ ID NO: 13) ; or
R₄₀-X₁CNTATCATQRLAEFLRHSSX₂₈STNVGSNTZ-CONH₂ (SEQ ID NO: 7)

10 wherein

X₁ is Lys or beta-Lys;

X₅ is Ala or Ser;

X₂₅ is Ala, Pro or hydroxyproline;

15 X₂₈ is Ser, Orn, hydroxyproline, N-acyl-hydroxyproline or isoacyl-hydroxyproline acylated at the alpha amine with a self-cleaving dipeptide element (A-B), wherein A is an amino acid and B is an N-alkylated amino acid;

X₂₉ is Ser, Pro or hydroxyproline;

Z is hydroxyproline or N-acyl-hydroxyproline;

R₂₀ is CONH₂; and

20 R₄₀ is a C16-C30 fatty acid, a C16-C30 phosphonic fatty acid, a C16-C30 diacid or a C16-C30 alkyl covalently linked to the alpha amine of Lys or the beta amine of beta-Lys, optionally via a spacer; wherein said first spacer is selected from the group consisting of a gamma glutamic acid, a gamma glutamic acid-gamma glutamic acid dipeptide, and a (gamma glutamic acid)_z-[COCH₂(OCH₂CH₂)_k-NH]_q-(gamma glutamic acid)_p, wherein z is 0 or 1, k is an integer selected from the range of 2-4 and q and p are independently an integer selected from the range of 0-4, or

25 R₄₀ is absent and X₁ is Lys or beta-Lys that is covalently linked to a branched polyethylene chain that comprises four branches of 5K each, or four branches of 10K where each branch is linked via a second spacer comprising
30 -[COCH₂(OCH₂CH₂)_k-NH]_q-(cysteine-S-S), wherein k is 2, and q is 1 or 2, optionally wherein k is 2.

In accordance with embodiment 52, an amylin analog of embodiment 51 is provided wherein X₁ is beta-Lys, acylated at the beta amine with a C16-C30 diacid or a C16-C30 phosphonic fatty acid, optionally via a first spacer selected from the group

consisting of a gamma glutamic acid, a gamma glutamic acid-gamma glutamic acid dipeptide.

In accordance with embodiment 53, an amylin analog of any one of embodiments 1-52 is provided wherein R_{40} - X_1 is beta-Lys, acylated at the beta amine
5 with $\gamma E-COC_{18}H_{36}CO_2H$ or $\gamma E-COC_{18}H_{36}PO_3H_2$.

In accordance with embodiment 54, an amylin analog of any one of embodiments 1-53 is provided wherein X_{25} is Ala, and Z is 4-hydroxyproline.

In accordance with embodiment 55, an amylin analog of any one of embodiments 1-54 is provided wherein X_1 is an amino acid in the D-stereoisomer
10 configuration.

In accordance with embodiment 56, an amylin analog of any one of embodiments 1-18 is provided wherein the amylin analog comprises the sequence of

R_{40} - X_1 CNTATCATQRLAEFLRHSSNNFGAILZSTNVGSNTZ-CONH₂
(SEQ ID NO: 3) or R_{40} - X_1 CNTATCATQRLAEFLRHSSZSTNVGSNTZ-CONH₂
15 (SEQ ID NO: 7

wherein

X_1 is Lys or beta-Lys;

Z is hydroxyproline or N-acyl-hydroxyproline; and

R_{40} is a C16-C20 fatty acid, a C16-C20 phosphonic fatty acid, or a
20 C16-C20 diacid covalently linked to the alpha amine of Lys or the beta amine of beta-Lys present at position 1, optionally via a spacer; wherein said first spacer is selected from the group consisting of a gamma glutamic acid or a gamma glutamic acid-gamma glutamic acid dipeptide; or

. R_{40} is absent and X_1 is Lys or beta-Lys that is covalently linked to a branched
25 polyethylene chain that comprises four branches of 5K each, or four branches of 10K where each branch is linked via a second spacer comprising

-[COCH₂(OCH₂CH₂)_k-NH]_q-(cysteine-S-S), wherein k is 2, and q is 1 or 2,
optionally wherein k is 2.

In accordance with embodiment 57, any one of embodiments 1-56 is provided
30 wherein X_1 is beta-Lys, optionally wherein beta-Lys in the D-stereoisomer configuration.

In accordance with embodiment 58, a peptide of embodiment 57 is provided wherein R_{40} is $\gamma E-COC_{18}H_{36}CO_2H$ covalently linked to the beta amine of said beta-Lys at position 1 of said peptide, optionally wherein Z is hydroxyproline.

In accordance with embodiment 59 one of embodiments 1-58 is provided wherein Z is 4-hydroxyproline or 3-hydroxyproline, optionally wherein Z is 4-hydroxyproline.

- In accordance with embodiment 60 a prodrug derivative of any one of the
- 5 DACRA peptides of embodiments 1-18 or any one of the conjugate derivatives of embodiments 19-42 or any one of the amylin analogs of embodiments 43-59 is provided wherein a self-cleaving dipeptide element (A-B) is linked to a primary amine of said DACRA peptide, said conjugate derivative or said amylin analog wherein A is an amino acid and B is an N-alkylated amino acid, optionally wherein
- 10 i) R_{40} is absent and the dipeptide element (A-B) is linked to the N-terminal alpha amine of the amylin analog peptide; or
- ii) the dipeptide element (A-B) is linked to the N-terminal amine of the incretin peptide of the conjugate derivative analogs; or
- iii) the amylin analog peptide comprises one or more of an isoacyl-
- 15 Thr at amino acid position 9, an isoacyl-Ser at amino acid position 19 or 29 or an isoacyl-hydroxyproline at amino acid position 28 or 29 of the peptide, relative to the amino acid sequence of SEQ ID NO: 1, further wherein the alpha amine of said isoacyl-Thr, isoacyl-Ser or isoacyl-hydroxyproline is covalently linked via an amide bond to a dipeptide element (A-B), wherein A is an amino acid and B is an N-
- 20 alkylated amino acid; or
- iv) i) and iii); or
- v) i) and ii).

In accordance with embodiment 61 a prodrug peptide derivative of any one of the DACRA peptides of embodiments 1-18 or the amylin analogs of any one of

25 embodiments 43-59 is provided wherein R_{40} is absent and the dipeptide element (A-B) is linked to the N-terminal alpha amine, and/or the peptide comprises one or more of an isoacyl-Thr at amino acid position 9, an isoacyl-Ser at amino acid position 19 or 29, an isoacyl-hydroxyproline at amino acid position 28 or 29 of the peptide, relative to the amino acid sequence of SEQ ID NO: 1, further wherein the alpha amine of said

30 isoacyl-Thr, isoacyl-Ser or isoacyl-hydroxyproline is covalently linked via an amide bond to a dipeptide element (A-B), wherein A is an amino acid and B is an N-alkylated amino acid.

In accordance with embodiment 62 a prodrug peptide derivative of any one of the conjugate derivatives of any one of embodiments 19-42 is provided wherein

the dipeptide element (A- B) is linked to the N-terminal alpha amine of the incretin peptide.

In accordance with embodiment 63 a prodrug peptide derivative of any one of the conjugate derivatives of any one of embodiments 19-42 and 62 is provided
5 wherein said dipeptide element (A-B) is covalently linked to the side chain of an amino acid at any one or more of positions 16, 17, 20, 24, 30 of the incretin peptide, relative to the sequence of SEQ ID NO: 1.

In accordance with embodiment 64 a prodrug of any one of embodiments 53-63 is provided wherein the peptide comprises an ester bond between any two adjacent
10 amino acids of SEQ ID NO: 1, with the proviso that when an ester bond is formed between two amino acids, then the second of the two amino acids is acylated with a C16-C30 fatty acid, a C16-C30 phosphonic fatty acid, or a diacid to stabilize the ester bond against hydrolytic cleavage of the ester, wherein an acylated self-cleaving dipeptide prodrug element is linked to the alpha amine of a hydroxylated amino acid,
15 including for example threonine or serine.

In accordance with embodiment 65 a prodrug peptide derivative of the DACRA peptides of any one of embodiments 1-18 is provided wherein

i) X_{28} is isoacyl-hydroxyproline, and
 X_{29} is selected from the group consisting of Ser, Orn, Lys,
20 hydroxyproline, N-alpha acyl-ornithine, N-alpha acetyl-Lys or N-acetyl-hydroxyproline, wherein an acylated self-cleaving dipeptide prodrug element (A-B) is linked to the alpha amine of the isoacyl-hydroxyproline at X_{28} , or

ii) X_{28} is selected from the group consisting of Ser, Orn, Lys, hydroxyproline, N-alpha acyl-ornithine, N-alpha acetyl-Lys or N-acetyl-
25 hydroxyproline, and

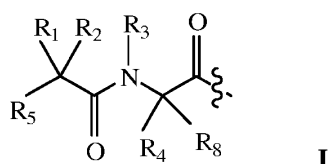
X_{29} is isoacyl-hydroxyproline, wherein an acylated self-cleaving dipeptide prodrug element (A-B) is linked to the alpha amine of the isoacyl-hydroxyproline at X_{29} , further wherein A is an amino acid acylated with a C16-C30 fatty acid, a C16-C30 phosphonic fatty acid, or a diacid and B is an N-alkylated
30 amino acid.

In accordance with embodiment 66 a prodrug isoacyl peptide derivative of embodiment 64 is provided wherein said prodrug peptide derivative comprises an isoacyl-Thr at position 9 of the peptide, relative to the amino acid sequence of SEQ ID NO: 1 or SEQ ID NO: 6, thus introducing an ester bond between the amino acids

at position 8 and 9, further wherein the alpha amine of said isoacyl-Thr is covalently linked via an amide bond to a dipeptide element (A-B), wherein A is an amino acid and B is an N-alkylated amino acid.

In accordance with embodiment 67, the prodrug derivative of any one of
 5 embodiments 60-66 is provided wherein the side chain of the first amino acid (A) of said dipeptide element is acylated with a C16-C30 fatty acid, a C16-C30 phosphonic fatty acid, or a C16-C30 diacid.

In accordance with embodiment 68, the prodrug derivative of any one of
 10 embodiments 60-67 is provided wherein the dipeptide prodrug element comprises the structure:



wherein

(I) R₁ and R₂ are independently selected from the group consisting of H, deuterium, C₁-C₁₈ alkyl, C₂-C₁₈ alkenyl, (C₁-C₁₈ alkyl)OH, (C₁-C₁₈ alkyl)SH, (C₂-C₃ alkyl)SCH₃, (C₁-C₄ alkyl)CONH₂, (C₁-C₄ alkyl)COOH, (C₁-C₄ alkyl)NH₂, (C₁-C₄ alkyl)NHC(NH₂⁺)NH₂, (C₀-C₄ alkyl)(C₃-C₆ cycloalkyl), (C₀-C₄ alkyl)(C₂-C₅ heterocyclic), (C₀-C₄ alkyl)(C₆-C₁₀ aryl)R₇, (C₁-C₄ alkyl)(C₃-C₉ heteroaryl), and C₁-C₁₂ alkyl(W₁₀)C₁-C₁₂ alkyl, wherein W₁₀ is a heteroatom selected from the group consisting of N, S and O, or R₁ and R₂ together with the atoms to which they are attached form a C₃-C₁₂ cycloalkyl;

R₃ is CD₃, C₁-C₁₈ alkyl;

20 R₄ and R₈ are each H or D;

R₅ is NHR₆, or R₅ and R₂ together with the atoms to which they are attached form a 4, 5 or 6 member heterocyclic ring;

R₆ is H or C₁-C₄ alkyl; and,

R₇ is selected from the group consisting of H, OH, halo, (C₁-C₇ alkyl), (C₂-C₇ alkenyl), OCF₃, NO₂, CN, NC, O(C₁-C₇ alkyl), CO₂H, CO₂(C₁-C₇ alkyl), NHR₆, aryl, and heteroaryl;

(II) R₁ and R₂ are independently selected from the group consisting of H, C₁-C₁₈ alkyl, C₂-C₁₈ alkenyl, (C₁-C₁₈ alkyl)OH, (C₁-C₁₈ alkyl)SH, (C₂-C₃ alkyl)SCH₃, (C₁-C₄ alkyl)CONH₂, (C₁-C₄ alkyl)COOH, (C₁-C₄ alkyl)NH₂, (C₁-C₄ alkyl)NHC(NH₂⁺)NH₂, (C₀-C₄ alkyl)(C₃-C₆ cycloalkyl), (C₀-C₄ alkyl)(C₂-C₅ heterocyclic), (C₀-C₄ alkyl)(C₆-C₁₀ aryl)R₇, (C₁-C₄ alkyl)(C₃-C₉ heteroaryl), and C₁-C₁₂ alkyl(W₁₀)C₁-C₁₂ alkyl, wherein W₁₀ is a heteroatom selected from the group consisting of N, S and O, or R₁ and R₂ together with the atoms to which they are attached form a C₃-C₁₂ cycloalkyl;

R₃ is C₁-C₁₈ alkyl;

10 R₄ is selected from the group consisting of CH₃, CH₂(C₁-C₁₀ alkyl), CH₂(C₂-C₁₀ alkenyl), CH₂(C₀-C₁₀ alkyl)OH, CH₂(C₀-C₁₀ alkyl)SH, CH₂(C₀-C₃ alkyl)SCH₃, CH₂(C₀-C₃ alkyl)CONH₂, CH₂(C₀-C₃ alkyl)COOH, CH₂(C₀-C₃ alkyl)NH₂, CH₂(C₀-C₃ alkyl)NHC(NH₂⁺)NH₂, CH₂(C₀-C₃ alkyl)(C₃-C₆ cycloalkyl), CH₂(C₀-C₃ alkyl)(C₂-C₅ heterocyclic), CH₂(C₀-C₃ alkyl)(C₆-C₁₀ aryl)R₇, CH₂(C₁-C₃ alkyl)(C₃-C₉ heteroaryl),
15 and CH₂(C₀-C₁₂ alkyl)(W₁₀)C₁-C₁₂ alkyl, wherein W₁₀ is a heteroatom selected from the group consisting of N, S and O, or R₄ and R₃ together with the atoms to which they are attached form a pyrrolidine, a 3,4-dehydropyrrolidine, a hydroxypyrrolidine, a piperidine or a hydroxypiperidine ring;

R₈ is H;

20 R₅ is NHR₆, or R₅ and R₂ together with the atoms to which they are attached form a 4, 5 or 6 member heterocyclic ring;

R₆ is H or C₁-C₄ alkyl; and,

R₇ is selected from the group consisting of H, OH, halo, (C₁-C₇ alkyl), (C₂-C₇ alkenyl), OCF₃, NO₂, CN, NC, O(C₁-C₇ alkyl), CO₂H, CO₂(C₁-C₇ alkyl), NHR₆, aryl, and heteroaryl;

(III) R₁ and R₂ are independently selected from the group consisting of H, C₁-C₁₈ alkyl, C₂-C₁₈ alkenyl, (C₁-C₁₈ alkyl)OH, (C₁-C₁₈ alkyl)SH, (C₂-C₃ alkyl)SCH₃, (C₁-C₄ alkyl)CONH₂, (C₁-C₄ alkyl)COOH, (C₁-C₄ alkyl)NH₂, (C₁-C₄ alkyl)NHC(NH₂⁺)NH₂, (C₀-C₄ alkyl)(C₃-C₆ cycloalkyl), (C₀-C₄ alkyl)(C₂-C₅ heterocyclic), (C₀-C₄ alkyl)(C₆-C₁₀ aryl)R₇, (C₁-C₄ alkyl)(C₃-C₉ heteroaryl), and C₁-C₁₂ alkyl(W₁₀)C₁-C₁₂ alkyl, wherein W₁₀ is a heteroatom selected from the group consisting of N, S and O, or R₁ and R₂ together with the atoms to which they are

attached form a C₃-C₁₂ cycloalkyl; or R₁ and R₂ together with the atoms to which they are attached form a C₃-C₁₂ cycloalkyl;

R₃ is C₁-C₁₈ alkyl;

R₄ is independently selected from the group consisting of CH(C₁-C₈ alkyl)₂,
 5 CH (C₂-C₈ alkenyl)₂, CH(C₁-C₈ alkyl)(OH), CH(C₁-C₈ alkyl)((C₁-C₈ alkyl)SH), and
 CH(C₁-C₃ alkyl)((C₁-C₈ alkyl)(NH₂)), or R₄ and R₃ together with the atoms to which
 they are attached form a pyrrolidine, a 3,4-dehydropyrrolidine, a hydroxypyrrolidine,
 a piperidine or a hydroxypiperidine ring;

R₈ is H;

10 R₅ is NHR₆, or R₅ and R₂ together with the atoms to which they are attached
 form a 4, 5 or 6 member heterocyclic ring;

R₆ is H or C₁-C₄ alkyl; and,

R₇ is selected from the group consisting of H, OH, halo, (C₁-C₇ alkyl), (C₂-C₇
 alkenyl), OCF₃, NO₂, CN, NC, O(C₁-C₇ alkyl), CO₂H, CO₂(C₁-C₇ alkyl), NHR₆, aryl,
 and heteroaryl;

(IV) R₁ and R₂ are independently selected from the group consisting of H,
 C₁-C₁₈ alkyl, C₂-C₁₈ alkenyl, (C₁-C₁₈ alkyl)OH, (C₁-C₁₈ alkyl)SH, (C₂-C₃ alkyl)SCH₃,
 15 (C₁-C₄ alkyl)CONH₂, (C₁-C₄ alkyl)COOH, (C₁-C₄ alkyl)NH₂, (C₁-C₄
 alkyl)NHC(NH₂⁺)NH₂, (C₀-C₄ alkyl)(C₃-C₆ cycloalkyl), (C₀-C₄ alkyl)(C₂-C₅
 heterocyclic), (C₀-C₄ alkyl)(C₆-C₁₀ aryl)R₇, (C₁-C₄ alkyl)(C₃-C₉ heteroaryl), and C₁-
 C₁₂ alkyl(W₁₀)C₁-C₁₂ alkyl, wherein W₁₀ is a heteroatom selected from the group
 consisting of N, S and O, or R₁ and R₂ together with the atoms to which they are
 20 attached form a C₃-C₁₂ cycloalkyl;

R₃ is CD₃, C₁-C₁₈ alkyl;

R₄ and R₈ are each H or D;

R₅ is NHR₆, or R₅ and R₂ together with the atoms to which they are attached
 form a 4, 5 or 6 member heterocyclic ring;

25 R₆ is H or C₁-C₄ alkyl; and,

R₇ is selected from the group consisting of H, OH, halo, (C₁-C₇ alkyl), (C₂-C₇
 alkenyl), OCF₃, NO₂, CN, NC, O(C₁-C₇ alkyl), CO₂H, CO₂(C₁-C₇ alkyl), NHR₆, aryl,
 and heteroaryl;

- (V) R_1 and R_2 are independently selected from the group consisting of H, C_1 - C_{18} alkyl, C_2 - C_{18} alkenyl, $(C_1$ - C_{18} alkyl)OH, $(C_1$ - C_{18} alkyl)SH, $(C_2$ - C_3 alkyl)SCH₃, $(C_1$ - C_4 alkyl)CONH₂, $(C_1$ - C_4 alkyl)COOH, $(C_1$ - C_4 alkyl)NH₂, $(C_1$ - C_4 alkyl)NHC(NH₂⁺)NH₂, $(C_0$ - C_4 alkyl)(C_3 - C_6 cycloalkyl), $(C_0$ - C_4 alkyl)(C_2 - C_5 heterocyclic), $(C_0$ - C_4 alkyl)(C_6 - C_{10} aryl) R_7 , $(C_1$ - C_4 alkyl)(C_3 - C_9 heteroaryl), and C_1 - C_{12} alkyl(W_{10}) C_1 - C_{12} alkyl, wherein W_{10} is a heteroatom selected from the group consisting of N, S and O, or R_1 and R_2 together with the atoms to which they are attached form a C_3 - C_{12} cycloalkyl; or R_1 and R_2 together with the atoms to which they are attached form a C_3 - C_{12} cycloalkyl;
- 10 R_3 is C_1 - C_{18} alkyl;
- R_4 is independently selected from the group consisting of CH(C_1 - C_8 alkyl)₂, CH(C_2 - C_8 alkenyl)₂, CH(C_1 - C_8 alkyl)(OH), CH(C_1 - C_8 alkyl)((C_1 - C_8 alkyl)SH), and CH(C_1 - C_3 alkyl)((C_1 - C_8 alkyl)(NH₂)) or R_4 and R_3 together with the atoms to which they are attached form a pyrrolidine, a 3,4-dehydropyrrolidine, a hydroxypyrrolidine,
- 15 a piperidine or a hydroxypiperidine ring;
- R_8 is H;
- R_5 is NHR₆, or R_5 and R_2 together with the atoms to which they are attached form a 4, 5 or 6 member heterocyclic ring;
- R_6 is H or C_1 - C_4 alkyl; and,
- R_7 is selected from the group consisting of H, OH, halo, $(C_1$ - C_7 alkyl), $(C_2$ - C_7 alkenyl), OCF₃, NO₂, CN, NC, O(C_1 - C_7 alkyl), CO₂H, CO₂(C_1 - C_7 alkyl), NHR₆, aryl, and heteroaryl; or
- 20 (VI) R_1 and R_2 are independently selected from the group consisting of H, C_1 - C_{18} alkyl, C_2 - C_{18} alkenyl, $(C_1$ - C_{18} alkyl)OH, $(C_1$ - C_{18} alkyl)SH, $(C_2$ - C_3 alkyl)SCH₃, $(C_1$ - C_4 alkyl)CONH₂, $(C_1$ - C_4 alkyl)COOH, $(C_1$ - C_4 alkyl)NH₂, $(C_1$ - C_4 alkyl)NHC(NH₂⁺)NH₂, $(C_0$ - C_4 alkyl)(C_3 - C_6 cycloalkyl), $(C_0$ - C_4 alkyl)(C_2 - C_5 heterocyclic), $(C_0$ - C_4 alkyl)(C_6 - C_{10} aryl) R_7 , $(C_1$ - C_4 alkyl)(C_3 - C_9 heteroaryl), and C_1 - C_{12} alkyl(W_{10}) C_1 - C_{12} alkyl, wherein W_{10} is a heteroatom selected from the group consisting of N, S and O, or R_1 and R_2 together with the atoms to which they are attached form a C_3 - C_{12} cycloalkyl; or R_1 and R_2 together with the atoms to which they are attached form a C_3 - C_{12} cycloalkyl;
- 25 R_3 is C_1 - C_{18} alkyl;

R₄ is independently selected from the group consisting of CH(C₁-C₈ alkyl)₂, CH (C₂-C₈ alkenyl)₂, CH(C₁-C₈ alkyl)(OH), CH(C₁-C₈ alkyl)((C₁-C₈ alkyl)SH), and CH(C₁-C₃ alkyl)((C₁-C₈ alkyl)(NH₂)) or R₄ and R₃ together with the atoms to which they are attached form a pyrrolidine, a 3,4-dehydropyrrolidine, hydroxypyrrolidine, a
 5 piperidine or a hydroxypiperidine ring;

R₈ is H;

R₅ is NHR₆, or R₅ and R₂ together with the atoms to which they are attached form a 4, 5 or 6 member heterocyclic ring;

R₆ is H or C₁-C₄ alkyl; and,

R₇ is selected from the group consisting of H, OH, halo, (C₁-C₇ alkyl), (C₂-C₇ alkenyl), OCF₃, NO₂, CN, NC, O(C₁-C₇ alkyl), CO₂H, CO₂(C₁-C₇ alkyl), NHR₆, aryl, and heteroaryl; or

10 (VII) R₁ is (C₁-C₄ alkyl)NH₂, optionally lLys or dLys;

R₂, R₈ are both H;

R₄ and R₃ together with the atoms to which they are attached form a pyrrolidine, a 3,4-dehydropyrrolidine, a hydroxypyrrolidine, a piperidine or a hydroxypiperidine ring; and

15 R₅ is NH₂., optionally wherein R₄ and R₃ together with the atoms to which they are attached form a hydroxy substituted pyrrolidine ring or a piperidine ring; or

VIII) R₁ is (C₁-C₄ alkyl)NH₂, optionally lLys or dLys, acylated with a C16-C20 fatty acid, a C16-C20 phosphonic fatty acid, or a C16-C20 diacid;

R₂ is H;

20 R₄ and R₈ are both D;

R₃ is CD₃; and

R₅ is NH₂; or

IX) R₁ is (C₁-C₄ alkyl)NH₂, optionally lLys or dLys, acylated with a C16-C20 fatty acid, a C16-C20 phosphonic fatty acid, or a C16-C20 diacid;

25 R₂, R₄ and R₈ are each H;

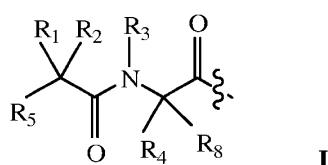
R₃ is CH₃; and

R₅ is NH₂.

In accordance with embodiment 69, the prodrug derivative any one of embodiments 60-68 is provided wherein the side chain of the first amino acid of
 30 dipeptide element A-B is acylated with a C16-C30 fatty acid, a C16-C30 phosphonic

fatty acid, or a C16-C30 diacid, optionally via a dipeptide element dipeptide spacer selected from the group consisting of a gamma glutamic acid, a gamma glutamic acid-gamma glutamic acid dipeptide, and a (gamma glutamic acid)_z-[COCH₂(OCH₂CH₂)_k-NH]_q-(gamma glutamic acid)_p, wherein z is 0 or 1, k is an integer selected from the range of 2-4 and q and p are independently an integer selected from the range of 0-4.

In accordance with embodiment 70, the prodrug derivative of any one of embodiments 60-69 is provided wherein the dipeptide element comprises the structure:



wherein

R₁ comprises a side chain of (C₁-C₄ alkyl)NH₂, optionally wherein a C16-C30 fatty acid, a C16-C30 phosphonic fatty acid, or a C16-C30 diacid is covalently linked to said side chain, optionally via a dipeptide element spacer, optionally wherein R₁ is Lys, acylated with a C16-C30 diacid;

R₂, is H or C₁-C₄ alkyl;

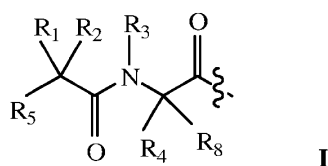
R₄ is H, D, C₁-C₄ alkyl, C₁-C₄ alkenyl, -(CH₂)_nCH₂OH or (C₁-C₄ alkyl)phenyl, wherein n is an integer selected from 0-2;

R₈ is H, D, or C₁-C₄ alkyl;

R₃ is CD₃, C₁-C₆ alkyl, or R₃ and R₄ together with the atoms to which they are attached form a pyrrolidine, a 3,4-dehydropyrrolidine, a hydroxypyrrolidine, a piperidine or a hydroxypiperidine ring; and

R₅ is NH₂, wherein said dipeptide spacer is selected from the group consisting of a gamma glutamic acid, a gamma glutamic acid-gamma glutamic acid dipeptide, and a (gamma glutamic acid)_z-[COCH₂(OCH₂CH₂)_k-NH]_q-(gamma glutamic acid)_p, wherein z is 0 or 1, k is an integer selected from the range of 2-4 and q and p are independently an integer selected from the range of 0-4.

In accordance with embodiment 71, the prodrug derivative of any one of embodiments 60-70 is provided wherein the dipeptide element comprises the structure:



wherein

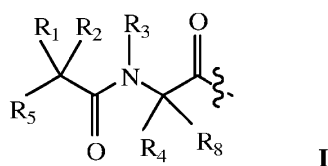
R₁ comprises a side chain of (C₄ alkyl)NH₂, having a C18-C20 fatty acid, a C18-C20 phosphonic fatty acid, or a C18-C20 diacid covalently linked to said side chain, optionally via a dipeptide spacer;

R₂, R₄ and R₈ are each H;

R₃ is C₁-C₆ alkyl; and

R₅ is NH₂, wherein the dipeptide spacer comprises - [COCH₂(OCH₂CH₂)_k-NH]_q-(gamma glutamic acid)_p, wherein k is 2, q is 0-2 and p is 0 or 1, with the proviso that q and p are not both 0.

In accordance with embodiment 72, the prodrug derivative of any one of the peptides of embodiments 60-71 is provided wherein said dipeptide spacer comprises the structure:



wherein

R₁ comprises a side chain of (C₄ alkyl)NH₂, having a C18-C20 fatty acid, a C18-C20 phosphonic fatty acid, or a C18-C20 diacid covalently linked to said side chain, optionally via said dipeptide spacer;

R₂ is H;

R₄ and R₈ are each H or D;

R₃ is CD₃ or C₁-C₆ alkyl; and

R₅ is NH₂, wherein said dipeptide spacer comprises - [COCH₂(OCH₂CH₂)_k-NH]_q-(gamma glutamic acid)_p, wherein k is 2, q is 1 or 2 and p is 0, 1 or 2.

In accordance with embodiment 73, the prodrug derivative any one of embodiments 60-72 is provided wherein said dipeptide spacer is

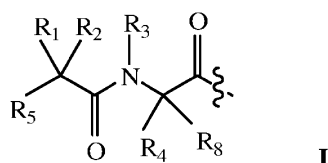
-[COCH₂(OCH₂CH₂)₂-NH]-(gamma glutamic acid)-, and the acyl group is a C18-C20 diacid or a C18-C20 phosphonic fatty acid.

In accordance with embodiment 74 the prodrug derivative of any one of
embodiments 60-73 is provided wherein the side chain of the first amino acid (A) of
said dipeptide element (A-B) is PEGylated with a straight chain or branched
polyethylene glycol chain having a molecular weight ranging from about 20k to about
5 40k, optionally via a dipeptide spacer comprising the structure:

i) $-\text{[COCH}_2(\text{OCH}_2\text{CH}_2)_k\text{-NH]}_q\text{-(alanine-triazole)}$, wherein k is 2, and q is 1 or
2, optionally wherein k is 2, or

ii) $-\text{[COCH}_2(\text{OCH}_2\text{CH}_2)_k\text{-NH]}_q\text{-(cysteine-S-S)}$, wherein k is 2, and q is 1 or 2,
optionally wherein k is 2.

10 In accordance with embodiment 75 the prodrug derivative of embodiment 74
is provided wherein said dipeptide element comprises the structure:



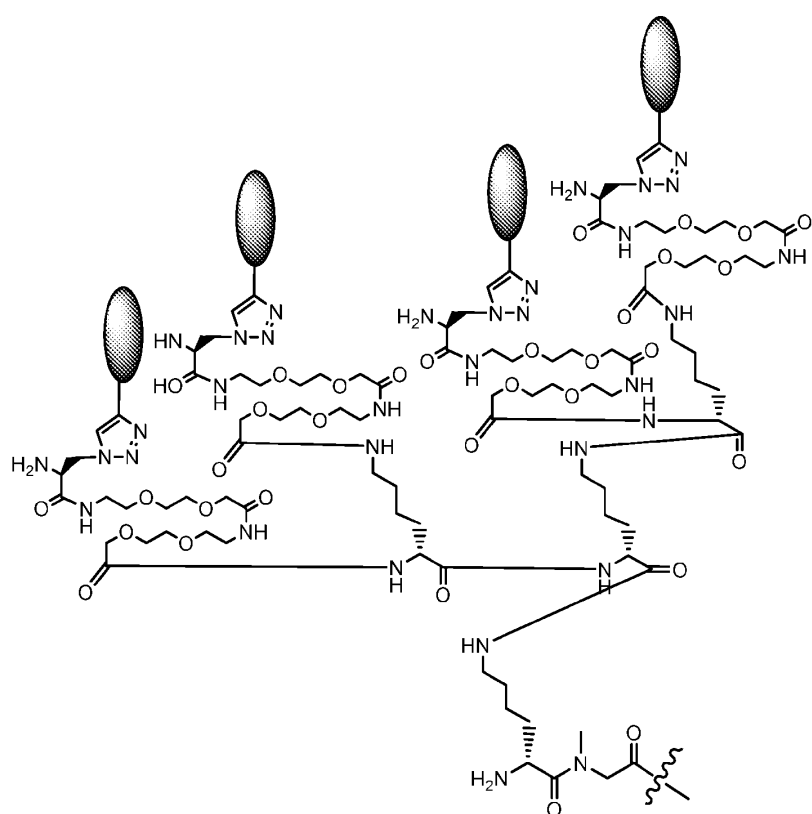
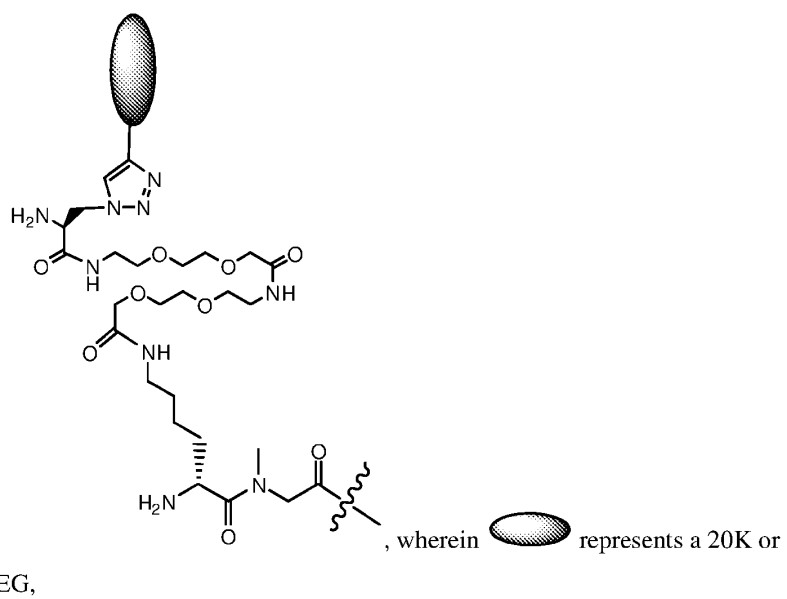
wherein

R_1 comprises a (C3-C4 alkyl)NH₂ side chain that has been pegylated;
15 R_2 , R_4 and R_8 are each H;
 R_3 is C₁-C₆ alkyl; and
 R_5 is NH₂.

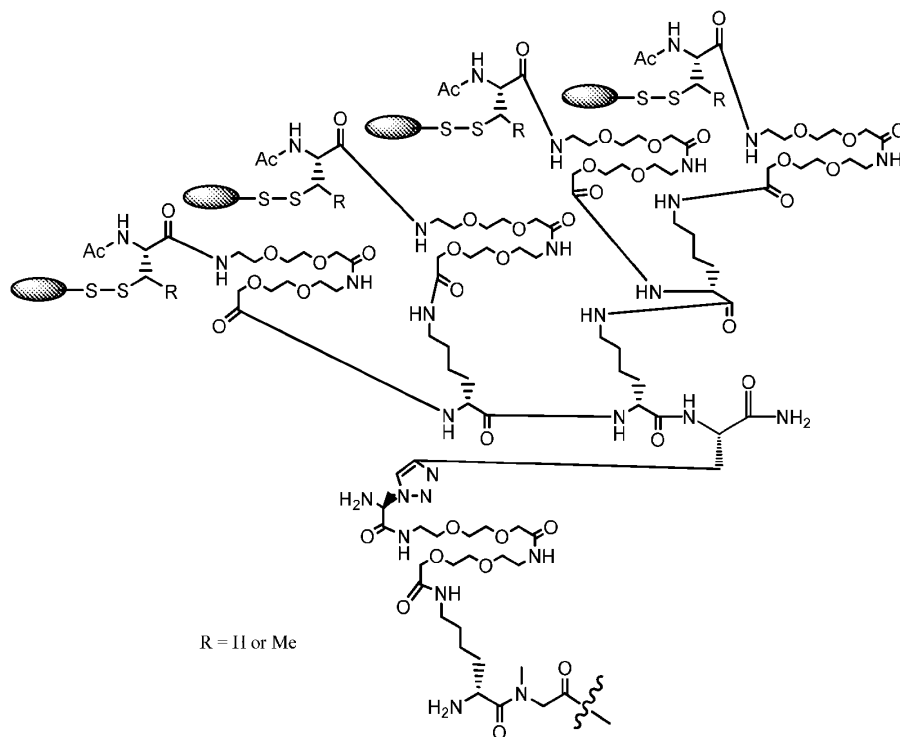
In accordance with embodiment 76 the prodrug derivative of embodiments 74
or 75 is provided wherein the first amino acid (A) of said dipeptide element (A-B) is
20 covalently linked to a branched polyethylene chain that comprises four branches of
5K each, or four branches of 10K where each branch is linked via a dipeptide spacer
comprising

$-\text{[COCH}_2(\text{OCH}_2\text{CH}_2)_k\text{-NH]}_q\text{-(cysteine-S-S)}$, wherein k is 2, and q is 1 or 2,
optionally wherein k is 2.

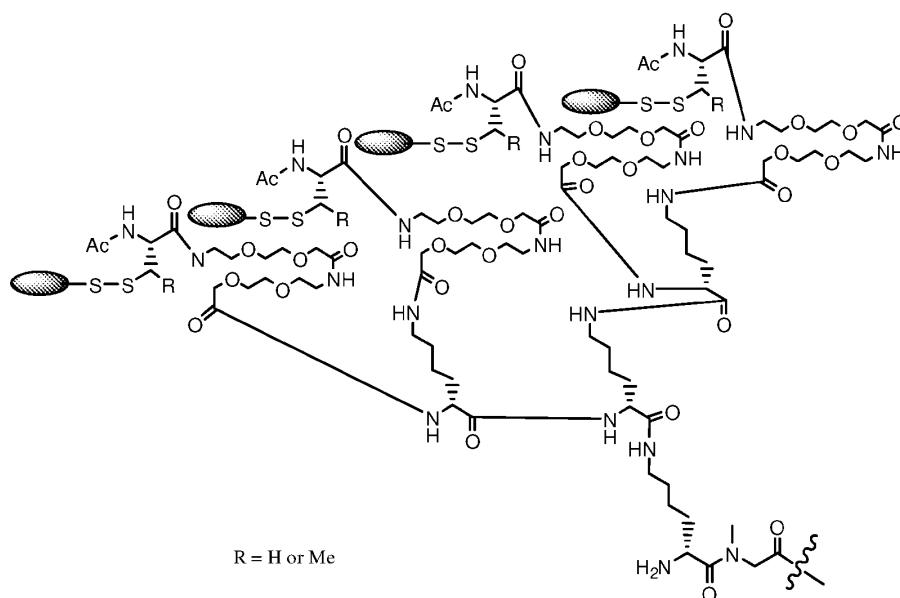
25 In accordance with embodiment 77 the prodrug derivative of any one of
embodiments 74-76 is provided wherein the first amino acid (A) of said dipeptide
element (A-B) comprises a structure selected from the group consisting of:



wherein  represents a 5K or 10K PEG,



, wherein  represents a 5K or 10K PEG and Ac = acyl group or



wherein  represents a 5K or 10K PEG and Ac = acyl group.

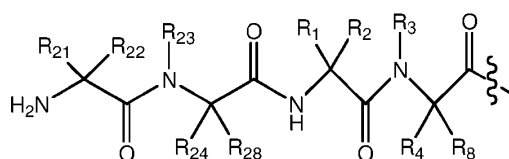
In accordance with embodiment 78 the prodrug derivative of any one of embodiments 60-77 is provided wherein

R_2, R_4 and R_8 are each H;

R₃ is C₁-C₆ alkyl; and

R₅ is NH₂.

In accordance with embodiment 79, the prodrug derivative any one of
10 embodiments 60-77 is provided wherein the dipeptide element comprises a sequential
dipeptide structure of



covalently linked via an amide bond to said prodrug derivative,
15 wherein

R₁ is (C₁-C₄ alkyl)NH₂, optionally linked to a C16-C30 fatty acid, a C16-C30 phosphonic fatty acid, or a C16-C30 diacid, optionally via a DD1 spacer;

R_{21} is $(C_1-C_4 \text{ alkyl})NH_2$, optionally linked to a C16-C30 fatty acid, a C16-C30 phosphonic fatty acid, or a C16-C30 diacid, optionally via a DD2 spacer;

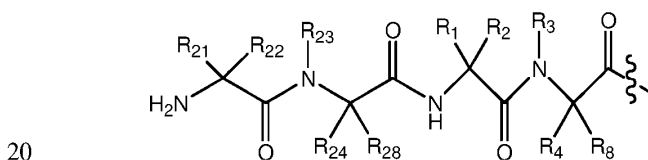
R_2 , and R_{22} , are independently H, or C₁-C₄ alkyl;

R_4 , R_8 , R_{24} and R_{28} are independently H, D, C₁-C₄ alkenyl, -
5 $(CH_2)_nCH_2OH$, or C₁-C₄ alkyl, wherein n is an integer selected from 0-2;

R_3 is C₁-C₆ alkyl, CD₃ or R_3 and R_4 together with the atoms to which they are attached form a pyrrolidine, a 3,4-dehydropyrrolidine, a hydroxypyrrolidine, a piperidine or a hydroxypiperidine ring; and

R_{23} is C₁-C₆ alkyl, CD₃ or R_{23} and R_{24} together with the atoms to
10 which they are attached form a pyrrolidine, a 3,4-dehydropyrrolidine, a hydroxypyrrolidine, a piperidine or a hydroxypiperidine ring, wherein said DD1 and DD2 spacers are independently selected from the group consisting of a gamma glutamic acid, a gamma glutamic acid-gamma glutamic acid dipeptide, and a (gamma glutamic acid)_z-[COCH₂(OCH₂CH₂)_k-NH]_q-(gamma glutamic acid)_p, wherein z is 0 or
15 1, k is an integer selected from the range of 2-4 and q and p are independently an integer selected from the range of 0-4.

In accordance with embodiment 80, the prodrug derivative of any one of embodiments 60-77 and 79 is provided wherein the sequential dipeptide comprises the structure:



wherein

R_1 is selected from an acylated amino acid having a side chain comprising $(C_1-C_4 \text{ alkyl})NH_2$ covalently linked to a C16-C30 fatty acid, a C16-C30 phosphonic fatty acid, or a C16-C30 diacid, optionally via a DD1 spacer;

25 R_{21} is selected from an acylated amino acid having a side chain comprising $(C_1-C_4 \text{ alkyl})NH_2$ covalently linked to a C16-C30 fatty acid, a C16-C30 phosphonic fatty acid, or a C16-C30 diacid, optionally via a DD2 spacer;

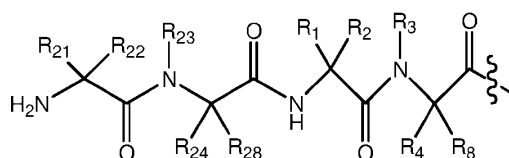
R_2 , and R_{22} are each H;

R_4 , R_8 , R_{24} and R_{28} are independently H or D;

30 R_3 and R_{23} are independently C₁-C₆ alkyl or CD₃; and wherein said DD1 and DD2 spacers are independently

$-\text{[COCH}_2(\text{OCH}_2\text{CH}_2)_k\text{-NH]}_q\text{-(gamma glutamic acid)}_p$, wherein k is 2, q is 1 or 2 and p is 0 or 1.

In accordance with embodiment 81, the prodrug derivative any one of embodiments 60-77 and 79-80 is provided wherein the sequential dipeptide comprises the structure:



wherein

R₁ is selected from an acylated amino acid having a side chain comprising (C₁-C₄ alkyl)NH₂ covalently linked to a C16-C30 fatty acid, a C16-C30 phosphonic fatty acid, or a C16-C30 diacid, optionally via a DD1 spacer;

R₂₁ is selected from an acylated amino acid having a side chain comprising (C₁-C₄ alkyl)NH₂ covalently linked to a C16-C30 fatty acid, a C16-C30 phosphonic fatty acid, or a C16-C30 diacid, optionally via a DD2 spacer;

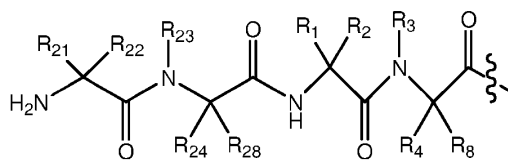
R₂, and R₂₂ are each H;

R₄, R₈, R₂₄ and R₂₈ are D;

R₃ and R₂₃ are each CD₃; wherein said DD1 spacer and DD2 spacer are independently $-\text{[COCH}_2(\text{OCH}_2\text{CH}_2)_k\text{-NH]}_q\text{-(gamma glutamic acid)}_p$, wherein k is 2, q is 1 or 2 and p is 0 or 1, optionally wherein k is 2 and q is 2 and p is 1.

In accordance with embodiment 82, the prodrug derivative of any one of embodiments 60-78 is provided wherein

said dipeptide element comprises a sequential dipeptide structure of



R₁ comprises a (C3-C4 alkyl)NH₂ side chain that has been pegylated, via a second spacer, with a straight chain or branched polyethylene glycol chain having a molecular weight ranging from about 20k to about 40k;

R₂₁ comprises (C3-C4 alkyl)NH₂ or a (C3-C4 alkyl)NH₂ side chain that has been pegylated, via a third spacer, with a straight chain or branched

polyethylene glycol chain having a molecular weight ranging from about 20k to about 40k;

R_2 , and R_{22} are independently H, or C₁-C₄ alkyl;

R_4 , R_8 , R_{24} and R_{28} are independently H, D, or C₁-C₄ alkyl;

5 R_3 is CD₃, C₁-C₆ alkyl, or R_3 and R_4 together with the atoms to which they are attached form a substituted or unsubstituted pyrrolidine, hydroxypyridine or piperidine ring; and

R_{23} is CD₃, C₁-C₆ alkyl, or R_{23} and R_{24} together with the atoms to which they are attached form a substituted or unsubstituted pyrrolidine,
10 hydroxypyridine or piperidine ring, wherein said second and third spacers are independently

-[COCH₂(OCH₂CH₂)_k-NH]_q-(alanine-triazole), wherein k is 2, and q is 1 or 2, optionally wherein k is 2, or -[COCH₂(OCH₂CH₂)_k-NH]_q-(cysteine-S-S), wherein k is 2, and q is 1 or 2, optionally wherein k is 2.

15 In accordance with embodiment 83, the prodrug derivative of embodiment 82 is provided wherein R_1 and optionally R_{21} are pegylated with a branched polyethylene glycol chain having the general structure of one of the branched structures provided in embodiment 77.

In accordance with embodiment 84, a pharmaceutical composition is provided
20 comprising the peptide of any one of embodiments 1 to 83 and a pharmaceutically acceptable carrier.

In accordance with embodiment 85 a method of reducing weight gain or inducing weight loss is provided, wherein the method comprises administering to a patient in need thereof a pharmaceutical composition of embodiment 84 in an amount
25 effective to reduce weight gain or induce weight loss.

In accordance with embodiment 86, the method of embodiment 85 is provided wherein the pharmaceutical composition is formulated for oral or inhalation delivery.

In accordance with embodiment 87, a calcitonin selective analog of amylin is provided, wherein the peptide comprises the sequence of

30 X₁CNTATCATQRLAEFLRHSSNNFGAIL- R_{20} (SEQ ID NO: 5) or

X₁CNTATCATQRLAEFLRHSSNNFGAILZSTNVGSN-COOH (SEQ ID NO: 2) or a peptide that differs from SEQ ID NO: 2 or SEQ ID NO: 5 by 1, 2, 3, 4 or 5 amino acid substitutions, optionally wherein the substitutions are conservative amino acid substitutions, wherein

X₁ is Lys, Orn, beta-Orn, or beta-Lys;

Z is hydroxyproline or N-acyl-hydroxyproline; and

R₂₀ is COOH or CONH₂

In accordance with embodiment 88, a calcitonin selective analog of amylin is
5 provided, wherein the peptide comprises the sequence of

X₁CNTATCATQRLAEFLRHSSNNFGAIL-R₂₀ (SEQ ID NO: 5))

wherein

X₁ is Lys or beta-Lys; and

R₂₀ is COOH or CONH₂.

10 In accordance with embodiment 89, the calcitonin selective analog of
embodiment 87 or 88 is provided wherein X₁ is beta-Lys; and Z is hydroxyproline.

In accordance with embodiment 90, the calcitonin selective analog of any one
of embodiments 87-89 is provided wherein Z is 4-hydroxyproline .

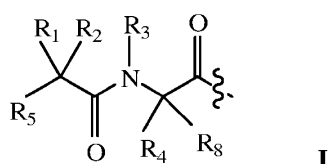
In accordance with embodiment 91 a prodrug derivative of the calcitonin
15 selective analog of any one of embodiments 87-90 is provided wherein a self-cleaving
acylated dipeptide (A-B) element is linked to the N-terminal amine of the calcitonin
selective analog, wherein A is an amino acid and B is an N-alkylated amino acid,
and/or the peptide comprises one or more of an isoacyl-Thr at amino acid position 9,
an isoacyl-Ser at amino acid position 19 or 29, or an isoacyl-hydroxyproline at amino
20 acid position 28 or 29 of the peptide, relative to the amino acid sequence of SEQ ID
NO: 1, further wherein the alpha amine of said isoacyl-Thr, isoacyl-Ser or isoacyl-
hydroxyproline is covalently linked via an amide bond to a dipeptide element (A-B),
wherein A is an amino acid and B is an N-alkylated amino acid.

In accordance with embodiment 92 a prodrug derivative of the calcitonin
25 selective analog of any one of embodiments 87-90 is provided wherein said prodrug
peptide derivative comprises an isoacyl-Thr at position 9 of the peptide, relative to the
amino acid sequence of SEQ ID NO: 1, thus introducing an ester bond between the
amino acids at position 8 and 9, further wherein the alpha amine of said isoacyl-Thr is
covalently linked via an amide bond to a dipeptide element (A-B), wherein A is an
30 amino acid and B is an N-alkylated amino acid.

In accordance with embodiment 93, the prodrug derivative any one of
embodiments 91-92 is provided wherein the side chain of the first amino acid of
dipeptide element A-B is acylated with a C16-C30 fatty acid, a C16-C30 phosphonic
fatty acid, or a C16-C30 diacid, optionally via a dipeptide spacer selected from the

group consisting of a gamma glutamic acid, a gamma glutamic acid-gamma glutamic acid dipeptide, and a (gamma glutamic acid)_z-[COCH₂(OCH₂CH₂)_k-NH]_q-(gamma glutamic acid)_p, wherein z is 0 or 1, k is an integer selected from the range of 2-4 and q and p are independently an integer selected from the range of 0-4.

- 5 In accordance with embodiment 94, the prodrug derivative of any one of embodiments 91-93 is provided wherein the dipeptide element comprises the structure:



wherein

- 10 R₁ comprises a side chain of (C₁-C₄ alkyl)NH₂, optionally wherein a C₁₆-C₃₀ fatty acid, a C₁₆-C₃₀ phosphonic fatty acid, or a C₁₆-C₃₀ diacid is covalently linked to said side chain, optionally via a dipeptide spacer, optionally wherein R₁ is Lys, acylated with a C₁₆-C₃₀ diacid;

R₂, is H or C₁-C₄ alkyl;

- 15 R₄ is H, D, C₁-C₄ alkyl, C₁-C₄ alkenyl, -(CH₂)_nCH₂OH or (C₁-C₄ alkyl)phenyl, wherein n is an integer selected from 0-2;

R₈ is H, D or C₁-C₄ alkyl;

- R₃ is CD₃, C₁-C₆ alkyl, or R₃ and R₄ together with the atoms to which they are attached form a pyrrolidine, a 3,4-dehydropyrrolidine, a hydroxypyrrolidine, 20 a piperidine or a hydroxypiperidine ring; and

- R₅ is NH₂, wherein said dipeptide spacer is selected from the group consisting of a gamma glutamic acid, a gamma glutamic acid-gamma glutamic acid dipeptide, and a (gamma glutamic acid)_z-[COCH₂(OCH₂CH₂)_k-NH]_q-(gamma glutamic acid)_p, wherein z is 0 or 1, k is an integer selected from the range of 2-4 and 25 q and p are independently an integer selected from the range of 0-4.

In accordance with embodiment 95, the prodrug derivative of embodiment 94 is provided wherein

R₂, is H;

R₄ is H, or D;

- 30 R₈ is H, or D;

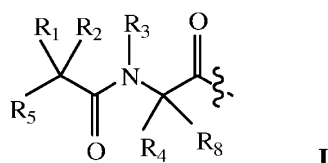
R₃ is CD₃, or C₁-C₆ alkyl.

In accordance with embodiment 96, the prodrug derivative of embodiment 91 or 92 is provided wherein the side chain of the first amino acid of dipeptide element A-B is PEGylated with a straight chain or branched polyethylene glycol chain having a molecular weight ranging from about 20k to about 40k, optionally via a second
 5 spacer comprising the structure:

i) $-\text{[COCH}_2\text{(OCH}_2\text{CH}_2\text{)}_k\text{-NH]}_q\text{-(alanine-triazole)}$, wherein k is 2, and q is 1 or 2, optionally wherein k is 2, or

ii) $-\text{[COCH}_2\text{(OCH}_2\text{CH}_2\text{)}_k\text{-NH]}_q\text{-(cysteine-S-S)}$, wherein k is 2, and q is 1 or 2, optionally wherein k is 2.

10 In accordance with embodiment 97, the prodrug derivative of embodiment 96 is provided wherein said dipeptide element comprises the structure:



wherein

R₁ comprises a (C3-C4 alkyl)NH₂ side chain that has been pegylated;

15 R₂, R₄ and R₈ are each H;

R₃ is C₁-C₆ alkyl; and

R₅ is NH₂.

In accordance with embodiment 98, the prodrug derivative of embodiment 96 or 97 is provided wherein R₁ and optionally R₂₁ are pegylated with a branched
 20 polyethylene glycol chain having the general structure of one of the branched structures provided in embodiment 77.

In accordance with embodiment 99, the prodrug derivative of embodiment 91 or 92 is provided wherein said dipeptide is a sequential dipeptide of any one of
 25 embodiments 79-83.

In accordance with 100, a pharmaceutical composition is provided comprising the calcitonin selective analog of any one of embodiments 87-99 and a pharmaceutically acceptable carrier.

In accordance with embodiment 101, a method of increasing bone growth, or preventing bone loss, in a subject in need thereof, is provided comprising

administering to said subject a calcitonin selective analog of any one of embodiments 87-99 and a pharmaceutically acceptable carrier.

In accordance with embodiment 102, an amylin analog is provided, comprising the structure of

5 $R_{40}-X_1CNTX_5TCATQRLAEFLRHSSNNFGX_{25}IL-W_1X_{28}X_{29}TNVGSNTZ-CONH_2$ (SEQ ID NO: 9),

$R_{40}-X_1CNTATCATQRLAEFLRHSS-W_1-X_{28}STNVGSNTZ-CONH_2$ (SEQ ID NO: 8) or a peptide that differs from SEQ ID NO: 7 or SEQ ID NO: 8 by 1, 2, 3, 4 or 5 amino acid substitutions, optionally wherein the substitutions are conservative

10 amino acid substitutions, wherein

X_1 is Lys, Orn, beta-Orn or beta-Lys;

X_5 is Ala or Ser;

X_{25} is Ala, Pro or hydroxyproline;

X_{28} is Ser, Orn, Lys, hydroxyproline, N-alpha acyl-ornithine, N-alpha

15 acyl-Lys or N-acyl-hydroxyproline;

X_{29} is Ser, Pro or hydroxyproline;

Z is pipecolic acid, Azetidine-2-carboxylic acid, hydroxyproline or N-acyl-hydroxyproline, optionally wherein Z is hydroxyproline or N-acyl-hydroxyproline; and

20 W_1 is a bifunctional linker comprising the structure

$[NH_2(CH_2CH_2O)_m(CH_2)_nCOOH]$, wherein m is an integer selected from the range of 1-20, and n is an integer selected from 1-3, optionally wherein m is 2, 4, 6, 8, or 10 and n is 1 or 2, optionally wherein m is 8 and n is 1;

25 R_{40} is absent, a C16-C30 fatty acid, a C16-C30 phosphonic fatty acid, a C16-C30 diacid group or a C16-C30 alkyl group covalently linked to the N-terminal alpha amine, the beta amine, or the side chain amine of the amino acid at position 1 (X_1), optionally via a first spacer; wherein said first spacer is selected from the group consisting of a gamma glutamic acid, gamma glutamic acid-gamma glutamic acid dipeptide, $[NH-(CH_2CH_2O)_m-COCH_2-]_n$ and a (gamma glutamic acid)z-
30 $[COCH_2(OCH_2CH_2)_k-NH]_q$ -(gamma glutamic acid)p, wherein m is an integer selected from the range of 2-4, n is an integer selected from the range of 1-10, z is 0 or 1, k is an integer selected from the range of 2-4 and q and p are independently an integer selected from the range of 0-4, optionally wherein

an incretin peptide is linked to the free amine of the amino acid at position 28 (i.e., if the amino acid at position 28 (X_{28}) is linked to W_1 via its alpha amine, then the incretin is linked to the side chain amine of the amino acid at position 28 (X_{28}) and vice versa), optionally via a conjugate linker, wherein said conjugate linker comprises
 5 the structure: $[NH_2(CH_2CH_2O)_m(CH_2)_nCOOH]$, wherein m is an integer selected from the range of 1-20 and n is 1 or 2, optionally wherein m is 2, 4, 6, 8 or 10 and n is 2, optionally wherein m is 8 and n is 2.

In accordance with embodiment 103, an amylin analog conjugate of embodiment 102 is provided, wherein X_1 is Lys or beta-Lys.

10 In accordance with embodiment 104, an amylin analog conjugate of embodiments 102 or 103 is provided, wherein X_{29} is hydroxyproline or serine.

In accordance with embodiment 105, an amylin analog conjugate of any one of embodiments 102-104 is provided, comprising the structure of R_{40} - X_1 CNTATCATQRLAEFLRHSS- W_1 - X_{28} STNVGSNTZ-CONH₂ (SEQ ID NO: 8) or
 15 a peptide that differs from SEQ ID NO: 8 by 1, 2, 3, 4 or 5 amino acid substitutions, optionally wherein the substitutions are conservative amino acid substitutions, wherein X_5 and X_{25} are both Ala.

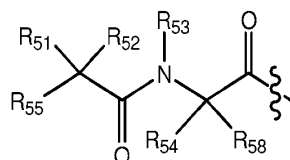
In accordance with embodiment 106, an amylin analog conjugate of any one of embodiments 102-105 is provided wherein

20 R_{40} is γ E-COC₁₈H₃₆CO₂H or γ E-COC₁₈H₃₆PO₃H₂ linked to the N-terminal alpha amine or beta amine of the amino acid at position 1 of said peptide.

In accordance with embodiment 107, an amylin analog conjugate of any one of embodiments 102-106 is provided wherein an incretin peptide is linked to the free amine of the amino acid at position 28 (i.e., if the amino acid at position 28 (X_{28}) is
 25 linked to W_1 via its alpha amine, then the incretin is linked to the side chain amine of the amino acid at position 28 (X_{28}) and vice versa), optionally via a conjugate linker, further wherein said W_1 and said conjugate spacer are each $NH_2(CH_2CH_2O)_mCH_2CH_2COOH$, wherein m is 8. In accordance with embodiment 108, the prodrug derivative of the conjugate derivatives of any one of embodiments
 30 19-42 is provided wherein a first dipeptide is covalently linked to the the alpha amine of the isoacyl-Thr at position 9 and a second dipeptide element (A-B), is linked to the N-terminal alpha amine or an amino acid side chain of the incretin peptide, wherein A is an amino acid and B is an N-alkylated amino acid.

In accordance with embodiment 109, the prodrug derivative of the conjugate derivatives of embodiment 108 is provided wherein the side chain of the first amino acid of the first and optional second dipeptide element A-B is acylated with a C16-C30 fatty acid, a C16-C30 phosphonic fatty acid, or a C16-C30 diacid, optionally via a dipeptide spacer selected from the group consisting of a gamma glutamic acid, a gamma glutamic acid-gamma glutamic acid dipeptide, and a (gamma glutamic acid)_z-[COCH₂(OCH₂CH₂)_k-NH]_q-(gamma glutamic acid)_p, wherein z is 0 or 1, k is an integer selected from the range of 2-4 and q and p are independently an integer selected from the range of 0-4.

In accordance with embodiment 110, the prodrug derivative of any one of embodiments 108-109 is provided wherein the first and second dipeptide elements independently comprises the structure:



wherein

R₅₁ comprises a side chain of (C₁-C₄ alkyl)NH₂, optionally wherein a C16-C30 fatty acid, a C16-C30 phosphonic fatty acid, or a C16-C30 diacid is covalently linked to said side chain, optionally via a dipeptide spacer, optionally wherein R₅₁ is Lys, acylated with a C16-C30 diacid;

R₅₂, is H or C₁-C₄ alkyl;

R₅₄ is H, D, C₁-C₄ alkyl, C₁-C₄ alkenyl, -(CH₂)_nCH₂OH or (C₁-C₄ alkyl)phenyl, wherein n is an integer selected from 0-2;

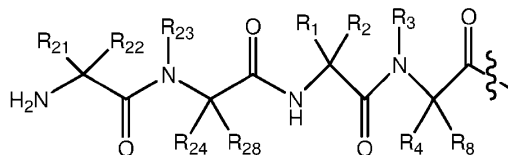
R₅₈ is H, D or C₁-C₄ alkyl;

R₅₃ is CD₃, C₁-C₆ alkyl, or R₅₃ and R₅₄ together with the atoms to which they are attached form a pyrrolidine, a 3,4-dehydropyrrolidine, a

hydroxypyrrolidine, a piperidine or a hydroxypiperidine ring; and

R₅₅ is NH₂, wherein said dipeptide spacer is selected from the group consisting of a gamma glutamic acid, a gamma glutamic acid-gamma glutamic acid dipeptide, and a (gamma glutamic acid)_z-[COCH₂(OCH₂CH₂)_k-NH]_q-(gamma glutamic acid)_p, wherein z is 0 or 1, k is an integer selected from the range of 2-4 and q and p are independently an integer selected from the range of 0-4.

In accordance with embodiment 111, the prodrug derivative of any one of embodiments 108-110 is provided wherein the first and second dipeptide elements independently comprise a sequential dipeptide having the structure:



5

wherein

R_1 is $(C_1-C_4 \text{ alkyl})NH_2$, optionally linked to a C16-C30 fatty acid, a C16-C30 phosphonic fatty acid, or a C16-C30 diacid, optionally via a DD1 spacer;

10 R_{21} is $(C_1-C_4 \text{ alkyl})NH_2$, optionally linked to a C16-C30 fatty acid, a C16-C30 phosphonic fatty acid, or a C16-C30 diacid, optionally via a DD2 spacer;

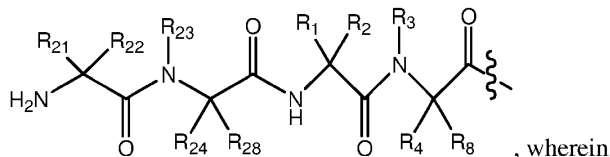
R_2 , and R_{22} are each H;

R_4 , R_8 , R_{24} and R_{28} are independently H or D;

15 R_3 and R_{23} are independently C_1-C_6 alkyl or CD_3 , wherein said DD1 and DD2 spacers are independently selected from the group consisting of a gamma glutamic acid, a gamma glutamic acid-gamma glutamic acid dipeptide, and a $(\text{gamma glutamic acid})_z-[COCH_2(OCH_2CH_2)_k-NH]_q-(\text{gamma glutamic acid})_p$, wherein z is 0 or 1, k is an integer selected from the range of 2-4 and q and p are independently an integer selected from the range of 0-4.

20 In accordance with embodiment 112, the prodrug derivative of embodiment 111 is provided wherein said sequential dipeptide is covalently linked via an amide bond to the alpha amine of the isoacyl-Thr at position 9, the isoacyl-Ser at position 19 or 29, or the isoacyl-hydroxyproline at position 28 or 29 of said prodrug derivative.

25 In accordance with embodiment 113, the prodrug derivative of any one of embodiments 108-112 is provided wherein said first and second dipeptide element independently comprise a sequential dipeptide structure of



R₁ comprises a (C3-C4 alkyl)NH₂ side chain that has been pegylated, via a second spacer, with a straight chain or branched polyethylene glycol chain having a molecular weight ranging from about 20k to about 40k;

R₂₁ comprises (C3-C4 alkyl)NH₂ or a (C3-C4 alkyl)NH₂ side chain
5 that has been pegylated, via a third spacer, with a straight chain or branched polyethylene glycol chain having a molecular weight ranging from about 20k to about 40k;

R₂, and R₂₂ are independently H, or C₁-C₄ alkyl;

R₄, R₈, R₂₄ and R₂₈ are independently H, D, or C₁-C₄ alkyl;

10 R₃ is CD₃, C₁-C₆ alkyl, or R₃ and R₄ together with the atoms to which they are attached form a substituted or unsubstituted pyrrolidine, hydroxypyridine or piperidine ring; and

R₂₃ is CD₃, C₁-C₆ alkyl, or R₂₃ and R₂₄ together with the atoms to which they are attached form a substituted or unsubstituted pyrrolidine,
15 hydroxypyridine or piperidine ring, wherein said second and third spacers are independently

-[COCH₂(OCH₂CH₂)_k-NH]_q-(alanine-triazole), wherein k is 2, and q is 1 or 2, optionally wherein k is 2, or -[COCH₂(OCH₂CH₂)_k-NH]_q-(cysteine-S-S), wherein k is 2, and q is 1 or 2, optionally wherein k is 2.

20 In accordance with embodiment 114, the prodrug derivative of embodiment 113 is provided wherein R₁ and optionally R₂₁ are pegylated with a branched polyethylene glycol chain having the general structure of one of the branched structures provided in embodiment 77.

In accordance with embodiment 115, the prodrug derivative of embodiment
25 113 is provided wherein both R₁ and R₂₁ are pegylated with a branched polyethylene glycol chain having the general structure of one of the branched structures provided in embodiment 77.

In accordance with embodiment 116, a pharmaceutical composition is provided comprising the peptide of any one of embodiments 108 to 115 and a
30 pharmaceutically acceptable carrier.

In accordance with embodiment 117 a method of reducing weight gain or inducing weight loss is provided, wherein the method comprises administering to a patient in need thereof a pharmaceutical composition of embodiment 116 in an amount effective to reduce weight gain or induce weight loss.

In accordance with embodiment 118 the method of embodiment 117 is provided wherein the pharmaceutical composition is formulated for oral or inhalation delivery.

5 EXAMPLES

Synthesis of Various Amylin/Calcitonin Co-agonists

• **MBX 5129**

MBX 5129 was assembled using a microwave assisted CEM Liberty Blue peptide synthesizer on a Rink Amide ProTide (LL) resin (0.1mmol). Fmoc-protected
10 amino acids were sequentially coupled using repetitive Ethyl cyanohydroxyiminoacetate (Oxyma)/N,N'-diisopropylcarbodiimide(DIC) activation. Fmoc protection groups were removed between couplings with 20% piperidine in DMF. The fatty acylation element (C20- γ E) was added directly to the peptide via the microwave synthesizer. The peptide was chemically removed from the synthetic resin
15 by treatment with a trifluoroacetic acid (TFA) solution containing 2.5% Triisopropylsilane (TIS), 2.5% 2,2'-(Ethylenedioxy)diethanethiol (DODT), 2.5 % anisole, and 2.5% H₂O at room temperature with gentle agitation for two hours. The resin was removed by filtration, and the peptide precipitated by addition of cold anhydrous diethyl ether (50 ml). The peptide precipitate was collected by
20 centrifugation and lyophilized to provide the crude product. Disulfide bond formation was achieved by dissolving the crude product in 50% ammonium acetate (pH 6.5) and 50% acetonitrile (ACN) and reacted, via gentle agitation with CLEAR-OXTM resin for two hours. The CLEAR-OXTM resin was removed by filtration and the newly formed product was lyophilized. The impure peptide was subjected to purification by
25 preparative reverse-phase HPLC column (Kinetex® 5 μ m C8 100 Å LC Column 250 X 21.2 mm, 10-50 % aqueous ACN (0.1% TFA), at a flow rate of 12 mL/min). The pure peptide was assessed by analytical LCMS and pooled fractions were lyophilized to provide the final product as a white fluffy solid.

30 • **MBX 5143**

MBX 5143 was assembled using a microwave assisted CEM Liberty Blue peptide synthesizer on a Rink Amide ProTide (LL) resin (0.1mmol) in analogous fashion as MBX 5129 with a single notable difference that pertained to synthesis of a

mid-sequence isoacyl ester bond. Following the addition of HYP²⁸ the resin was removed from the microwave at which time the α -amine of Hyp²⁸ was acetylated utilizing acetic anhydride and N,N-diisopropylethylamine (DIPEA). Utilizing the O-acyl isopeptide method Leu²⁷ was manually esterified to HYP²⁸ utilizing DIC and 4-dimethylaminopyridine (DMAP). The remaining amino acid residues (β Lys¹ through Ile²⁶), were coupled using an ABI-433A peptide synthesizer so as not disturb the newly formed ester bond. The fatty acylation element (C20- γ E) was manually coupled to the peptide utilizing Oxyma/DIC coupling protocols. The remainder of the synthesis was analogous to MBX 5129.

10

- **MBX 5164**

MBX 5164 was assembled using a microwave-assisted CEM Liberty Blue peptide synthesizer on a Rink Amide ProTide (LL) resin (0.1mmol) in analogous fashion as MBX 5129 with a single notable difference that pertained to a mid-sequence mini-peg insertion. Fmoc-protected amino acids were sequentially coupled using repetitive Oxyma)/DIC activation. Fmoc-PEG2 (Fmoc-NH(CH₂CH₂O)₂CH₂COOH) was coupled in comparable manner as that of the other amino acids following the coupling of Hyp²⁸. The remaining amino acid residues (β Lys¹ through Leu²⁷), were coupled using the microwave synthesizer. The fatty acylation element (C20- γ E) was manually coupled to the peptide utilizing Oxyma/DIC coupling protocols. The remainder of the synthetic protocol was analogous to MBX 5129.

15

20

- **MBX 5166**

MBX 5166 was assembled using a microwave-assisted CEM Liberty Blue peptide synthesizer on a Rink Amide ProTide (LL) resin (0.1mmol) in analogous fashion as MBX 5129 with a single notable difference that pertained to a mid-sequence use of ornithine and a mini-peg spacer. Following the addition of S²⁹ the resin was removed from the microwave at which time ORN²⁸ protected in Mtt form was manually coupled using OXYMA/DIC. The α -amine of ORN²⁸ was acetylated utilizing acetic anhydride. The MTT protecting group on the δ -amine (delta) of ornithine was removed utilizing 30% hexafluoroisopropyl alcohol (HFIPA) in dichloromethane (DCM). The remaining amino acid residues (β Lys¹ through Leu²⁷),

25

30

as well as the PEG2 spacer ((Fmoc-NH(CH₂CH₂O)₂CH₂COOH), was coupled to the δ-amine using the microwave peptide synthesizer. The remainder of the synthetic protocol was analogous to MBX 5129.

5 • **MBX 5175**

MBX 5175 was assembled using a microwave-assisted CEM Liberty Blue peptide synthesizer on a Rink Amide ProTide (LL) resin (0.1mmol) in analogous fashion as MBX 5166 with a single notable difference that pertained to a mid-sequence use mini-peg 8 spacer and addition of a GLP-1 agonist peptide. Following
10 the addition of S²⁹ the resin was removed from the microwave at which time ORN²⁸ was manually coupled using OXYMA/DIC. The α-amine of ORN₂₈ was acylated, with a PEG8 spacer (Fmoc-NH (CH₂CH₂O)₈CH₂COOH) followed by an addition of the semaglutide sequence without fatty acylation, utilizing the microwave synthesizer. Of note, His¹ was manually coupled in the Boc-protected form to “cap”
15 the sequence. The MTT protecting group on the δ-amine of ornithine was removed utilizing 30% HFIPA in DCM. The remaining amino acid residues (βLys¹ through Leu²⁷), as well as a PEG8 spacer, was coupled to the δ-amine using the microwave peptide synthesizer. The remainder of the synthetic protocol was analogous to MBX 5129

20

Assessment of cleavage half-life

Peptide prodrugs were dissolved in PBS buffer and adjusted to obtain a pH of 7.4. The resulting solution was incubated at 37 °C. Aliquots were taken at designed time points and analyzed by LC-MS. The analysis was performed using an Agilent
25 1260 Infinity instrument with Phenomenex Kinetex C8 2.6μ 100A (75×4.6 mm) column. Flow rate of 1mL/min and a gradient of 10% - 80% acetonitrile in water, with 0.1% trifluoroacetic acid over 10 min. Data was collected using absorption at 214 nm. Positive mode MS data were obtained with an Agilent 6120 Quadrupole LC/MS. The concentration of prodrug and drug were determined by their relative
30 peak areas. The first order dissociation rate constant of the prodrug was determined by plotting the logarithm of the concentration of the prodrug at various time points. The slope of this plot provides the rate constant ‘k’. The half-life of the cleavage was calculated based upon the formula $t_{1/2} = 0.693/k$.

***In Vitro* Receptor Pharmacology**

- **Elisa-Based Reporter Assay for DACRA Agonism**

The bioactivity of each DACRA analog to promote intracellular phosphorylation of ERK1/2 was determined in HEK293 cells which stably overexpress one of the three human amylin receptors (hAmyR1-3) or the human calcitonin receptor (hCTR). The degree of activity was determined by cell-based ELISA assays for downstream enzyme activation.

The engineered hAMYRs and hCTR cells were maintained in high glucose DMEM medium (Gibco, Life Technologies, Grand Island, NY) supplemented with 10% Bovine Growth Serum supplement Calf (Cat: SH30541.03, HyClone) in a CO₂ incubator at 37°C. Cells growing in logarithmic phase were seeded in 96-well plates and incubated overnight at 37°C. After 2 hours starvation in DMEM basal medium containing 0.2% ovalbumin (Cat: A5503-25G, Sigma-Aldrich), cells were treated with serially diluted DACRA peptide analogs for 5 minutes at 37°C, followed by fixation in 10% neutralized formalin (HT501128, Sigma-Aldrich) at RT for 20min. The fixed cells were respectively probed with rabbit anti-phospho-ERK1/2 primary antibody (Cat: 700012, Invitrogen) in PBS with 3% blotting blocker (Cat: 170-6404, Bio-Rad) at 4°C overnight and anti-rabbit IgG-HRP conjugated second antibody (Cat: HA008, R&D systems) in PBS at RT for 1 hour. The color was developed in TMB single solution (Cat: 002023, Fisher Scientific) and signal was measured with the 2014 multilabel reader (Perkin Elmer). All experimental results were analyzed by using a 3-parameter non-linear regression curve in GraphPad Prism 7. Potency was determined by comparative analysis of relative EC₅₀ values. Each experiment was repeated at least three times with each sample assayed in duplicate.

25

- **Luciferase-Based Reporter Assay for GLP-1 cAMP Agonism**

The ability of each peptide analog or prodrug to induce cAMP was measured in a firefly luciferase-based reporter assay. The cAMP production that is induced is directly proportional to the peptide binding and activation of the specific single overexpressed receptor. HEK293 cells co-transfected with the human GLP-1 and a luciferase gene linked to a cAMP inducible responsive element were employed for the bioassay.

30

The cells were serum-deprived by culturing 16 hours in Dulbecco Minimum Essential Medium (Gibco, Life Technologies, Grand Island, NY) supplemented with 0.3% FetalClone III (HyClone, Logan, UT) and then incubated with serial dilutions of the peptide analogs or prodrugs for 5 hours at 37° C., 5% CO₂ in 96-well “Costar 3610” Assay plates (Corning, Kennebunk, ME). At the end of the incubation, 50 µL of Steady-Lite Plus luminescence substrate reagent (PerkinElmer, Waltham, MA) were added to each well.

The plate was shaken briefly at 600 rpm, incubated for four minutes and light output was measured on an EnSpire Alpha Multi-mode Plate Reader (PerkinElmer, Waltham, MA). The effective 50% concentrations (EC₅₀) were calculated using Origin 2019b software (OriginLab, Northampton, MA), and the effective concentration 50 (EC₅₀) was determined by sigmoidal fitting. Potency was determined by comparative analysis of relative EC₅₀ values. Each experiment was repeated at least three times with each sample assayed in duplicate.

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***In Vivo* Rat Pharmacology**

Peptide-based drug and prodrug candidates were tested for their *in vivo* effects in diet induced obese rats (DIO Sprague Dawley strain). Groups of six experimental rats with initial body weight that varied at start of treatment in the 600-700g range were subcutaneously injected with vehicle or test peptides at a specified dose that varied between 0.5-20 nmol/kg. The rats varied in age from 6 to 12 months and had been on a high fat diet for approximately two months prior to treatment. Body weights and food consumption were typically measured each morning and no less frequently than every other day in repeat dose experimental protocols.

The SD rats were obtained from Harlan Laboratories. They were single housed as approved by and performed according to the guidelines of the Institutional Animal Care and Use Committee of the University of Cincinnati on a 12:12 h light-dark cycle at 22 °C with free access to food and water.

Assessment of Pharmacokinetics: Measuring Plasma Peptide Concentration by LCMS

Following peptide administration, blood was collected on EDTA-coated tubes from 4 rats or two cynomolgus monkeys at specified times. The injected dose volume was 0.8ml/kg for rats and 0.3 mL/kg for monkeys. Individual dose volumes were

calculated based on the animals' most recently recorded body weight. Blood samples were maintained at $5 \pm 3^{\circ}\text{C}$ (wet ice) and centrifuged at $5 \pm 3^{\circ}\text{C}$ within 1 hour for the collection of each blood sample. The resultant plasma was transferred to a tube and then stored under conditions set to maintain $-75 \pm 15^{\circ}\text{C}$ until analysis.

- 5 Standard curve samples were prepared by serial dilution with species specific plasma on the day of analysis. Aliquots (40 μl) of standard curve and study samples were transferred to a 96-well plate and mixed with 160 μl of Methanol:Acetonitrile (ACN) (1:1, v/v) internal standard solution. After 10 minutes of centrifugation, supernatants were diluted 2-fold with acidified (0.1% formic acid) ACN:Water
10 (3:1, v/v) and analyzed by LC-MS/MS.

- A Shimadzu CBM-20A Nexera UPLC system and a CTC PAL autosampler comprised the front end of the LC-MS/MS system. Chromatography was based on an Accurcore C8 Column, 2.6 μm , 2.1 mm X 30 mm (Thermo 17226-032130) and a binary gradient program of 0.1% formic acid (aq) and 0.1% formic acid in
15 ACN. Mobile phase solvent A consisted of micro filtered water:formic acid (1000:1 v/v), and solvent B consisted of ACN:formic acid (1000:1). The flow rate was 0.8 ml/min., the column temperature was ambient, and the injection volume was 5 μl . The two needles rinses were ACN:Water (25:75, v/v) and ACN:Isopropanol:Acetone in 0.1% formic acid (5:4:1, v/v/v). The gradient cycle
20 started at 15% B (concentration) with a linear increase to 65% B in 0.75 minute. The column was cleaned with 98% B for 0.25 min. and returned to initial %B during acquisition time of 1.20 minute. The total cycle time for each injection, including re-equilibration of initial % B, was approximately 3 minutes. The first 0.3 min. of each run was diverted to waste.
- 25 Mass spectrometric data were generated using Analyst software controlling a Sciex API 6500 plus triple-quadrupole mass spectrometer (Model 5060743-J) in positive ionization mode.

What is claimed is:

1. An amylin analog having agonist activity at the calcitonin receptor, said peptide comprising the sequence

$R_{40}-X_1CNTX_5TCAX_9QRLAEFLRHX_{19}SNNFGX_{25}IL-W_1-X_{28}X_{29}TNVGSNTZ-R_{20}$ (SEQ ID NO: 1),

$R_{40}-X_1CNTX_5TCATQRLAEFLRHSS-W_1-X_{28}X_{29}TNVGSNTZ-R_{20}$ (SEQ ID NO: 6)

or a peptide that differs from SEQ ID NO: 1 or SEQ ID NO: 6 by 1 or 2 amino acid substitutions, optionally wherein the substitutions are conservative amino acid substitutions, wherein

X_1 is Lys, Orn, beta-Orn, or beta-Lys, in either the L-stereoisomer configuration or D-stereoisomer configuration;

X_5 is Ala or Ser;

X_9 is Thr, isoacyl-Thr, N-acetyl-Thr or isoacyl-Thr acylated at the alpha amine with a self-cleaving dipeptide element (A-B), wherein A is an amino acid and B is an N-alkylated amino acid;

W_1 is absent or comprises the structure $NH_2(CH_2CH_2O)_m(CH_2)_nCOOH$, wherein m is an integer selected from the range of 1-20 and n is 1 or 2, optionally wherein m is 2, 4, 6, 8 or 10 and n is 1 or 2, optionally wherein m is 8 and n is 2;

X_{19} is Ser, N-acetyl-Ser or isoacyl-Ser acylated at the alpha amine with a self-cleaving dipeptide element (A-B), wherein A is an amino acid and B is an N-alkylated amino acid;

X_{25} is Ala, Pro or hydroxyproline;

X_{28} is selected from the group consisting of Ser, Orn, Lys, hydroxyproline, N-alpha acyl-ornithine, N-alpha acetyl-Lys, N-acetyl-hydroxyproline and isoacyl-hydroxyproline acylated at the alpha amine with a self-cleaving dipeptide element (A-B), wherein A is an amino acid and B is an N-alkylated amino acid;

X_{29} is selected from the group consisting of Ser, Orn, Lys, hydroxyproline, N-alpha acyl-ornithine, N-alpha acetyl-Lys, N-acetyl-hydroxyproline, isoacyl-Ser acylated at the alpha amine with a self-cleaving dipeptide element (A-B), wherein A is an amino acid and B is an N-alkylated amino acid, and

isoacyl-hydroxyproline acylated at the alpha amine with a self-cleaving dipeptide element (A-B), wherein A is an amino acid and B is an N-alkylated amino acid;

Z is pipecolic acid, Azetidine-2-carboxylic acid, hydroxyproline or N-acyl-hydroxyproline;

R₂₀ is COOH or CONH₂; and

R₄₀ is absent, or a C16-C30 fatty acid, a C16-C30 phosphonic fatty acid, a C16-C30 diacid, a C16-C30 alkyl, or a straight chain or branched polyethylene glycol chain having a molecular weight ranging from about 20k to about 40k, covalently linked to the alpha amine, the beta amine, or the side chain amine of the amino acid at position 1 (X₁), optionally via a first spacer; wherein said first spacer comprises a gamma glutamic acid, a gamma glutamic acid-gamma glutamic acid dipeptide, or a (gamma glutamic acid)_z-[COCH₂(OCH₂CH₂)_k-NH]_q-(gamma glutamic acid)_p, wherein z is 0 or 1, k is an integer selected from the range of 2-4 and q and p are independently an integer selected from the range of 0-4.

2. The peptide of claim 1 having amylin/calcitonin co-agonist activity, wherein R₂₀ is CONH₂.

3. The peptide of claim 2 wherein

X₁ is Lys, dLys, Orn, beta-Orn or beta-Lys acylated with a C16-C30 fatty acid, a C16-C30 phosphonic fatty acid, or a C16-C30 diacid.

4. The peptide of claim 2 wherein

X₁ is beta-Lys, acylated at the beta amine with a C16-C30 diacid or a C16-C30 phosphonic fatty acid, optionally via a first spacer selected from gamma glutamic acid, a gamma glutamic acid-gamma glutamic acid dipeptide.

5. The peptide of claim 4 wherein X₁ is Lys, dLys, Orn, beta-Orn or beta-Lys acylated with γ ECOC₁₈H₃₆CO₂H, or γ ECOC₁₈H₃₆PO₃H₂; X₂₅ is Ala; and Z is 4-hydroxyproline.

6. The peptide of claim 2 wherein

R₄₀ is absent; and

X₁ is Lys, dLys, Orn, beta-Orn or beta-Lys covalently linked via a second spacer to a straight chain or branched polyethylene glycol chain having a molecular weight ranging from about 20k to about 40k, wherein said second spacer comprises

i) -[COCH₂(OCH₂CH₂)_k-NH]_q-(alanine-triazole), wherein k is 2, and q is 1 or 2, optionally wherein k is 2, or

ii) $-\text{[COCH}_2(\text{OCH}_2\text{CH}_2)_k\text{-NH]}_q\text{-(cysteine-S-S)}$, wherein k is 2, and q is 1 or 2, optionally wherein k is 2.

7. The peptide of claim 6 wherein X_1 is Lys or beta-Lys that is covalently linked to a branched polyethylene chain that comprises four branches of 5K each, or four branches of 10K where each branch is linked via a second spacer comprising $-\text{[COCH}_2(\text{OCH}_2\text{CH}_2)_k\text{-NH]}_q\text{-(cysteine-S-S)}$, wherein k is 2, and q is 1 or 2, optionally wherein k is 2.

8. The peptide of any one of claims 1-7 wherein X_{28} is selected from the group consisting of Orn, Lys, hydroxyproline, N-alpha acyl-ornithine, N-alpha acetyl-Lys and N-acetyl-hydroxyproline, and X_{29} is serine.

9. The peptide of any one of claims 1-8 wherein W_1 is $\text{NH}_2(\text{CH}_2\text{CH}_2\text{O})_m(\text{CH}_2)_n\text{COOH}$, wherein m is 2, 4, 6, 8, 10 or 16 and n is 1 or 2, optionally wherein m is 8 and n is 2.

10. The peptide of any one of claims 1-9 is provided wherein X_5 and X_{25} are both Ala and Z is hydroxyproline.

11. The peptide of any one of claims 1-10 is provided wherein X_9 is Thr and X_{19} is Ser.

12. A conjugate derivative of the peptide of claim 9 wherein an incretin peptide is linked to the free amine of the amino acid at X_{28} of said peptide, optionally via a conjugate spacer, said conjugate linker comprising the structure: $\text{NH}_2(\text{CH}_2\text{CH}_2\text{O})_m(\text{CH}_2)_n\text{COOH}$, wherein m is 6, 8 or 10 and n is 1 or 2, further wherein

i) if the amino acid at (X_{28}) is linked to W_1 via the alpha amine of said amino acid, then the incretin peptide is linked to the side chain amine of the amino acid at X_{28} ;

ii) if the amino acid at X_{28} is linked to W_1 via the side chain amine of said amino acid, then the incretin peptide is linked to the alpha amine of the amino acid at X_{28} .

13. The conjugate derivative of claim 12 wherein X_{28} is Lys, further wherein the alpha amine of said Lys is linked to W_1 and the side chain amine is linked to the carboxy terminus of said incretin peptide via said conjugate linker.

14. The conjugate derivative of claim 12 or 13 wherein said incretin peptide is selected from glucagon, GLP-1, GIP or any analog thereof wherein said analog exhibits agonist activity at any one of the glucagon, GIP and GLP-1 receptors, or co-agonist activity at the GIP and GLP-1 receptors, or exhibits tri-agonist activity at the glucagon, GIP and GLP-1 receptors.

15. The conjugate derivative of claim 12 or 13 wherein the incretin peptide comprises the sequence of

YX₂X₃GTX₆X₇SDYSIX₁₃LX₁₅KIAQX₂₀AFVQWLIAGGPSSGAPPPS-

R₂₀ (SEQ ID NO: 14) or

YX₂X₃GTFTSDYSIX₁₃LX₁₅KX₁₇AQX₄₀AFVQWLLEGGPSSGAPPPS-

R₂₀ (SEQ ID NO: 15)

wherein

X₂ is Aib;

X₃ is Glu or Gln;

X₆ is alpha methylated Phe or Phe;

X₇ is isoacyl-Thr, Thr, or isoacyl-Thr acylated at the alpha amine with a self-cleaving dipeptide disclosed herein dipeptide element (A-B), wherein A is an amino acid and B is an N-alkylated amino acid;

X₁₃ is Aib or alpha methylated Leu;

X₁₅ is Glu or Asp;

X₄₀ is Aib;

R₂₀ is CONH₂; and

X₁₇ and X₂₀ are independently an amino acid comprising a (C1-C4 alkyl)NH₂ side chain that has been acylated with a C16-C20 acyl group, a C16-C20 phosphonic fatty acid, or a C16-C20 alkyl group, optionally via a conjugate spacer; wherein the conjugate spacer comprises the structure: -[COCH₂(OCH₂CH₂)_kNH]_q-(gamma glutamic acid)_p-; wherein k is 2, p is 1 or 2 and q is an integer selected from 1, 2 or 4, optionally wherein k is 2, p is 1 and q is 2. I

16. The conjugate derivative of claim 15 wherein

i) the incretin peptide comprises the sequence of SEQ ID NO: 14 wherein X₂ is Aib, X₃ is Glu, X₆ is alpha methylated Phe, X₇ is said acylated isoacyl-Thr or Thr; X₁₃ is Aib, X₁₅ is Glu, X₂₀ is Lys acylated via its side chain with (COCH₂(OCH₂CH₂)₂NH)₂-γE-COC₁₈H₃₆CO₂H, or Lys acylated via its side chain with (COCH₂(OCH₂CH₂)₂NH)₂-γE-COC₁₈H₃₆PO₃H₂ and R₂₀ is CONH₂;

ii) the incretin peptide comprises the sequence of SEQ ID NO: 15 wherein X_2 is Aib, X_3 is Gln, X_{13} is alpha methylated Leu, and X_{15} is Asp; X_{17} is Lys acylated via its side chain with $(\text{COCH}_2(\text{OCH}_2\text{CH}_2)_2\text{NH})_2\text{-}\gamma\text{E-COC}_{18}\text{H}_{36}\text{CO}_2\text{H}$ or Lys acylated via its side chain with $(\text{COCH}_2(\text{OCH}_2\text{CH}_2)_2\text{NH})_2\text{-}\gamma\text{E-COC}_{18}\text{H}_{36}\text{PO}_3\text{H}_2$, X_{40} is Aib and R_{20} is CONH_2 .

17. The conjugate derivative of claim 12 or 13 wherein said incretin peptide is selected from Semaglutide (SEQ ID NO: 10), tirzepatide (SEQ ID NO: 11), retatrutide (SEQ ID NO: 12) or known analog thereof.

18. The conjugate derivative of claim 12 comprising the structure of $R_{40}\text{-}X_1\text{CNTATCATQRLAEFLRHSSNNFGAIL-W}_1\text{-}X_{28}\text{STNVGSNTZ-CONH}_2$ (SEQ ID NO: 4), or

$R_{40}\text{-}X_1\text{CNTATCATQRLAEFLRHSS-W}_1\text{-}X_{28}\text{STNVGSNTZ-CONH}_2$ (SEQ ID NO: 8), wherein

X_1 is Lys, Orn, beta-Orn or beta-Lys;

X_{28} is Lys or Orn linked to W_1 via the alpha amine, or the side chain amine, of the Lys or Orn;

Z is hydroxyproline;

W_1 is a bifunctional linker comprising the structure $\text{NH}_2(\text{CH}_2\text{CH}_2\text{O})_m(\text{CH}_2)_n\text{COOH}$, wherein m is an integer selected from the range of 1-20 and n is 1 or 2, optionally wherein m is 2, 4, 6, 8 or 10 and n is 1 or 2, optionally wherein m is 8 and n is 2; and

R_{40} is absent, or a C16-C30 fatty acid, a C16-C30 phosphonic fatty acid, a C16-C30 diacid group or a C16-C30 alkyl group covalently linked to the N-terminal alpha amine, the beta amine, or the side chain amine of the amino acid at position 1 (X_1), optionally via a first spacer; wherein said first spacer comprises gamma glutamic acid, a gamma glutamic acid-gamma glutamic acid dipeptide, or (gamma glutamic acid) $_z$ - $[\text{COCH}_2(\text{OCH}_2\text{CH}_2)_k\text{-NH}]_q$ -(gamma glutamic acid) $_p$, wherein z is 0 or 1, k is an integer selected from the range of 2-4 and q and p are independently an integer selected from the range of 0-4, optionally wherein R_{40} is $\gamma\text{E-COC}_{18}\text{H}_{36}\text{CO}_2\text{H}$; and

an incretin peptide linked to a primary amine of the amino acid at position 28, via a conjugate spacer, wherein said conjugate spacer comprises a bifunctional linker comprising the structure $\text{NH}_2(\text{CH}_2\text{CH}_2\text{O})_m(\text{CH}_2)_n\text{COOH}$, wherein m is an integer

selected from the range of 1-20 and n is 1 or 2, optionally wherein m is 2, 4, 6, 8 or 10 and n is 1 or 2, optionally wherein m is 8 and n is 2.

19. The conjugate derivative of claim 18 comprising the structure of $R_{40}-X_1\text{CNTATCATQRLAEFLRHSS}-W_1-X_{28}\text{STNVGSNTZ}-\text{CONH}_2$ (SEQ ID NO: 8).

wherein

X_1 is beta-Lys;

X_{28} is Orn or Lys linked to W_1 via the alpha amine or the side chain amine of the Orn or Lys;

Z is hydroxyproline;

W_1 and said conjugate spacer independently comprise a structure of $\text{NH}_2(\text{CH}_2\text{CH}_2\text{O})_m\text{CH}_2\text{CH}_2\text{COOH}$, wherein m is 4, 6, 8 or 10, optionally wherein m is 8;

R_{40} is a C16-C20 phosphonic fatty acid, or a C16-C20 diacid group covalently linked to the beta amine of beta-Lys via a first spacer; wherein said first spacer comprises gamma glutamic acid; and said incretin peptide is linked to the amino acid at position X_{28} via said conjugate spacer.

20. The conjugate derivative of claim 19 wherein

X_{28} is Lys linked to W_1 via the alpha amine or the side chain amine of said Lys; and.

R_{40} is $\gamma\text{E}-\text{COC}_{18}\text{H}_{36}\text{CO}_2\text{H}$ or $\gamma\text{E}-\text{COC}_{18}\text{H}_{36}\text{PO}_3\text{H}_2$ linked to the beta amine of said beta-Lys.

21 The conjugate derivative of claim 19 wherein said incretin peptide comprises the sequence of

$\text{YX}_2\text{X}_3\text{GTX}_6\text{X}_7\text{SDYSIX}_{13}\text{LX}_{15}\text{KIAQX}_{20}\text{AFVQWLIAGGPSSGAPPPS}-R_{20}$ (SEQ ID NO: 14), wherein X_2 is Aib, X_3 is Glu, X_6 is alpha methylated Phe, X_7 is said acylated isoacyl-Thr or Thr; X_{13} is Aib, X_{15} is Glu, X_{20} is Lys acylated via its side chain with $(\text{COCH}_2(\text{OCH}_2\text{CH}_2)_2\text{NH})_2-\gamma\text{E}-\text{COC}_{18}\text{H}_{36}\text{CO}_2\text{H}$, or Lys acylated via its side chain with $(\text{COCH}_2(\text{OCH}_2\text{CH}_2)_2\text{NH})_2-\gamma\text{E}-\text{COC}_{18}\text{H}_{36}\text{PO}_3\text{H}_2$ and R_{20} is CONH_2 ; or

$\text{YX}_2\text{X}_3\text{GTFTSDYSIX}_{13}\text{LX}_{15}\text{KX}_{17}\text{AQX}_{40}\text{AFVQWLLEGGPSSGAPPPS}-R_{20}$ (SEQ ID NO: 15) wherein X_2 is Aib, X_3 is Gln, X_{13} is alpha methylated Leu, and X_{15} is Asp; X_{17} is Lys acylated via its side chain with $(\text{COCH}_2(\text{OCH}_2\text{CH}_2)_2\text{NH})_2-\gamma\text{E}-\text{COC}_{18}\text{H}_{36}\text{CO}_2\text{H}$ or Lys acylated via its side chain with $(\text{COCH}_2(\text{OCH}_2\text{CH}_2)_2\text{NH})_2-\gamma\text{E}-\text{COC}_{18}\text{H}_{36}\text{PO}_3\text{H}_2$, X_{40} is Aib and R_{20} is CONH_2 .

22. The conjugate derivative of claim 19 wherein said incretin peptide is selected from Semaglutide (SEQ ID NO: 10), tirzepatide (SEQ ID NO: 11), retatrutide (SEQ ID NO: 12) or known analogs thereof.

23. The conjugate derivative of claim 21 or 22 wherein W_1 and the conjugate spacer are both $NH_2(CH_2CH_2O)_8CH_2CH_2COOH$.

24. A peptide having amylin/calcitonin co-agonist activity, said peptide comprising the sequence of

$R_{40}-X_1CNTATCATQRLAEFLRHSSNNFGAILZSTNVGSNTZ-CONH_2$ (SEQ ID NO: 3); or

$R_{40}-X_1CNTATCATQRLAEFLRHSSZSTNVGSNTZ-CONH_2$ (SEQ ID NO: 7)

wherein

X_1 is Lys or beta-Lys;

Z is hydroxyproline or N-acyl-hydroxyproline; and

R_{40} is a C16-C30 fatty acid, a C16-C30 phosphonic fatty acid, a C16-C30 diacid group or a C16-C30 alkyl group covalently linked to the alpha amine of Lys or the beta amine of beta-Lys present at position 1, optionally via a first spacer; wherein said first spacer is selected from the group consisting of a gamma glutamic acid, a gamma glutamic acid-gamma glutamic acid dipeptide, and a (gamma glutamic acid) $_z$ -[COCH₂(OCH₂CH₂) $_k$ -NH] $_q$ -(gamma glutamic acid) $_p$, wherein z is 0 or 1, k is an integer selected from the range of 2-4 and q and p are independently an integer selected from the range of 0-4.

25. The peptide of claim 24, wherein X_1 is beta-Lys.

26. The peptide of any one of claims 1-25, wherein X_1 is an amino acid in the D-configuration.

27. The peptide of any one of claims 24-26,

R_{40} is $\gamma E-COC_{18}H_{36}CO_2H$, or $\gamma E-COC_{18}H_{36}PO_3H_2$, linked to the N-terminal alpha amine or beta amine of the amino acid at position 1 of said peptide.

28. The peptide of claim 27 wherein Z is 3-hydroxyproline.

29. An amylin analog having amylin/calcitonin co-agonist activity, wherein said amylin analog comprises the sequence of

$R_{40}-X_1CNTX_5TCATQRLAEFLRHSSNNFGX_{25}ILX_{28}X_{29}TNVGSNTZ-R_{20}$
(SEQ ID NO: 13) ; or

$R_{40}-X_1CNTATCATQRLAEFLRHSSZSTNVGSNTZ-CONH_2$ (SEQ ID NO: 7)

wherein

X_1 is Lys or beta-Lys;

X_5 is Ala or Ser;

X_{25} is Ala, Pro or hydroxyproline;

X_{28} is Ser, Orn, hydroxyproline or N-acyl-hydroxyproline;

X_{29} is Ser, Pro or hydroxyproline;

Z is hydroxyproline or N-acyl-hydroxyproline;

R_{20} is $CONH_2$; and

R_{40} is a C16-C30 fatty acid, a C16-C30 phosphonic fatty acid, a C16-C30 diacid or a C16-C30 alkyl covalently linked to the alpha amine of Lys or the beta amine of beta-Lys, optionally via a first spacer; wherein said first spacer is selected from the group consisting of a gamma glutamic acid, a gamma glutamic acid-gamma glutamic acid dipeptide, and a $(\text{gamma glutamic acid})_z-[\text{COCH}_2(\text{OCH}_2\text{CH}_2)_k-\text{NH}]_q-(\text{gamma glutamic acid})_p$, wherein z is 0 or 1, k is an integer selected from the range of 2-4 and q and p are independently an integer selected from the range of 0-4, or

R_{40} is absent and X_1 is Lys or beta-Lys that is covalently linked to a branched polyethylene chain that comprises four branches of 5K each, or four branches of 10K where each branch is linked via a second spacer comprising $-\text{[COCH}_2(\text{OCH}_2\text{CH}_2)_k-\text{NH}]_q-(\text{cysteine-S-S})$, wherein k is 2, and q is 1 or 2, optionally wherein k is 2.

30. The amylin analog of claim 29 wherein

X_1 is beta-Lys, acylated at the beta amine with a C16-C30 diacid or a C16-C30 phosphonic fatty acid, optionally via a first spacer selected from the group consisting of a gamma glutamic acid, and a gamma glutamic acid-gamma glutamic acid dipeptide; and

$R_{40}-X_1$ is beta-Lys, acylated at the beta amine with $\gamma\text{E-COC}_{18}\text{H}_{36}\text{CO}_2\text{H}$ or $\gamma\text{E-COC}_{18}\text{H}_{36}\text{PO}_3\text{H}_2$.

31. A calcitonin selective analog of amylin, said peptide comprising the sequence of

$X_1CNTATCATQRLAEFLRHSSNNFGAIL-R_{20}$ (SEQ ID NO: 5) or

X₁CNTATCATQRLAEFLRHSSNNFGAILZSTNVGSN-COOH (SEQ ID NO: 2) or a peptide that differs from SEQ ID NO: 2 or SEQ ID NO: 5 by 1 or 2 amino acid substitutions, optionally wherein the substitutions are conservative amino acid substitutions, wherein

X₁ is Lys, Orn, beta-Orn, or beta-Lys;

Z is hydroxyproline or N-acyl-hydroxyproline; and

R₂₀ is COOH or CONH₂.

32. The calcitonin selective analog of claim 31, wherein the peptide comprises the sequence of

X₁CNTATCATQRLAEFLRHSSNNFGAIL-R₂₀ (SEQ ID NO: 5)

wherein

X₁ is Lys or beta-Lys; and

R₂₀ is COOH or CONH₂.

33. The calcitonin selective analog of claim 32 wherein X₁ is beta-Lys; and Z is hydroxyproline.

34. The calcitonin selective agonist of claim 32 or 33 wherein R₄₀ is γE-COC₁₈H₃₆CO₂H or γE-COC₁₈H₃₆PO₃H₂ linked to the N-terminal alpha amine or beta amine, or the side chain amine of the amino acid at position 1 of said peptide.

35. A prodrug derivative of any one of the amylin analogs of claims 1-11 or 24-30, the calcitonin selective agonist of claims 31-34 or any one of the conjugate derivatives of claims 12-23 wherein a self-cleaving dipeptide element (A-B) is linked to a primary amine of said amylin analog or conjugate derivative thereof wherein A is an amino acid and B is an N-alkylated amino acid.

36. The prodrug derivative of claim 35 wherein

i) R₄₀ is absent and the dipeptide element (A-B) is linked to the N-terminal alpha amine of the amylin analog peptide; or

ii) the dipeptide element (A-B) is linked to the N-terminal amine of the incretin peptide of the conjugate derivative analogs; or

iii) the amylin analog peptide comprises one or more of an isoacyl-Thr at amino acid position 9, an isoacyl-Ser at amino acid position 19 or 29, or an isoacyl-hydroxyproline at amino acid position 28 or 29 of the peptide, relative to the amino acid sequence of SEQ ID NO: 1, further wherein the alpha amine of said

isoacyl-Thr, isoacyl-Ser or isoacyl-hydroxyproline is covalently linked via an amide bond to a dipeptide element (A-B), wherein A is an amino acid and B is an N-alkylated amino acid; or

iv) i) and iii); or

v) i) and ii).

37. A prodrug derivative of a conjugate derivative of any one of claims 12-23 wherein the dipeptide element (A-B) is linked to the N-terminal alpha amine of the incretin peptide, optionally wherein R₄₀ is absent and a second dipeptide element (A-B) is linked to the N-terminal alpha amine.

38. The prodrug derivative of claim 31 wherein a dipeptide element (A-B) is covalently linked to the side chain of an amino acid at any one of positions 16, 17, 20, 24 and 30 of the incretin peptide, relative to the sequence of SEQ ID NO: 11.

39. A prodrug peptide derivative of the DACRA peptides of any one of claims 1-11 is provided wherein

i) X₂₈ is isoacyl-hydroxyproline, and

X₂₉ is selected from the group consisting of Ser, Orn, Lys, hydroxyproline, N-alpha acyl-ornithine, N-alpha acetyl-Lys and N-acetyl-hydroxyproline, wherein an acylated self-cleaving dipeptide prodrug element (A-B) is linked to the alpha amine of the hydroxyproline at X₂₈, or

ii) X₂₈ is selected from the group consisting of Ser, Orn, Lys, hydroxyproline, N-alpha acyl-ornithine, N-alpha acetyl-Lys and N-acetyl-hydroxyproline, and

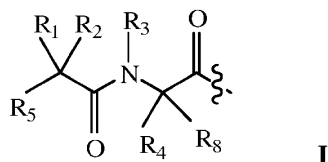
X₂₉ is isoacyl-hydroxyproline, wherein an acylated self-cleaving dipeptide prodrug element (A-B) is linked to the alpha amine of the hydroxyproline at X₂₉,

further wherein A is an amino acid acylated with a C16-C30 fatty acid, a C16-C30 phosphonic fatty acid, or a C16-C30 diacid and B is an N-alkylated amino acid.

40. The prodrug derivative of any one of claims 35-39, wherein the side chain of the first amino acid (A) of said dipeptide element is acylated with a C16-C30 fatty acid, a C16-C30 phosphonic fatty acid, or a C16-C30 diacid, optionally via a dipeptide spacer, wherein said dipeptide spacer comprises a gamma glutamic acid, a gamma glutamic acid-gamma glutamic acid dipeptide, and a (gamma glutamic acid)_z-[COCH₂(OCH₂CH₂)_k-NH]_q-(gamma glutamic acid)_p, wherein z is 0 or 1, k is an

integer selected from the range of 2-4 and q and p are independently an integer selected from the range of 0-4.

41. The prodrug derivative of any one of claims 35-40 wherein the dipeptide prodrug element comprises the structure:



wherein

(I) R_1 and R_2 are independently selected from the group consisting of H, deuterium, C_1 - C_{18} alkyl, C_2 - C_{18} alkenyl, $(C_1$ - C_{18} alkyl)OH, $(C_1$ - C_{18} alkyl)SH, $(C_2$ - C_3 alkyl)SCH₃, $(C_1$ - C_4 alkyl)CONH₂, $(C_1$ - C_4 alkyl)COOH, $(C_1$ - C_4 alkyl)NH₂, $(C_1$ - C_4 alkyl)NHC(NH₂⁺)NH₂, $(C_0$ - C_4 alkyl)(C_3 - C_6 cycloalkyl), $(C_0$ - C_4 alkyl)(C_2 - C_5 heterocyclic), $(C_0$ - C_4 alkyl)(C_6 - C_{10} aryl) R_7 , $(C_1$ - C_4 alkyl)(C_3 - C_9 heteroaryl), and C_1 - C_{12} alkyl(W_{10}) C_1 - C_{12} alkyl, wherein W_{10} is a heteroatom selected from the group consisting of N, S and O, or R_1 and R_2 together with the atoms to which they are attached form a C_3 - C_{12} cycloalkyl;

R_3 is CD₃, C_1 - C_{18} alkyl;

R_4 and R_8 are each H or D;

R_5 is NHR₆, or R_5 and R_2 together with the atoms to which they are attached form a 4, 5 or 6 member heterocyclic ring;

R_6 is H or C_1 - C_4 alkyl; and,

R_7 is selected from the group consisting of H, OH, halo, $(C_1$ - C_7 alkyl), $(C_2$ - C_7 alkenyl), OCF₃, NO₂, CN, NC, O(C_1 - C_7 alkyl), CO₂H, CO₂(C_1 - C_7 alkyl), NHR₆, aryl, and heteroaryl;

(II) R_1 is $(C_1$ - C_4 alkyl)NH₂, optionally ILys or dLys, acylated with a C_{16} - C_{20} fatty acid, a C_{16} - C_{20} phosphonic fatty acid, or a C_{16} - C_{20} diacid, optionally via a dipeptide spacer;

R_2 and R_8 are both H;

R_3 is CD₃, C_1 - C_{18} alkyl;

R₄ is H or R₄ and R₃ together with the atoms to which they are attached form a pyrrolidine, a 3,4-dehydropyrrolidine, a hydroxypyrrolidine, a piperidine or a hydroxypiperidine ring; and

R₅ is NH₂; or

(III) R₁ is (C₁-C₄ alkyl)NH₂, optionally lLys or dLys, acylated with a C16-C20 fatty acid, a C16-C20 phosphonic fatty acid, or a C16-C20 diacid, optionally via a dipeptide spacer;

R₂ is H;

R₄ and R₈ are both D;

R₃ is CH₃ or CD₃; and

R₅ is NH₂; or

(IV) R₁ is (C₁-C₄ alkyl)NH₂, optionally lLys or dLys, acylated with a C16-C20 fatty acid, a C16-C20 phosphonic fatty acid, or a C16-C20 diacid, optionally via a dipeptide spacer;

R₂, R₄ and R₈ are each H;

R₃ is CH₃; and

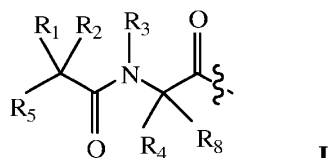
R₅ is NH₂.

42. The prodrug derivative of any one of claims 35-38, wherein the side chain of the first amino acid (A) of said dipeptide element is PEGylated with a straight chain or branched polyethylene glycol chain having a molecular weight ranging from about 20k to about 40k, optionally via a dipeptide spacer comprising the structure:

i) -[COCH₂(OCH₂CH₂)_k-NH]_q-(alanine-triazole), wherein k is 2, and q is 1 or 2, optionally wherein k is 2, or

ii) -[COCH₂(OCH₂CH₂)_k-NH]_q-(cysteine-S-S), wherein k is 2, and q is 1 or 2, optionally wherein k is 2.

43. The prodrug derivative of claim 42 wherein said dipeptide element comprises the structure:



wherein

R_1 comprises a (C3-C4 alkyl)NH₂ side chain that has been pegylated, optionally via a dipeptide spacer;

R_2 , R_4 and R_8 are each H;

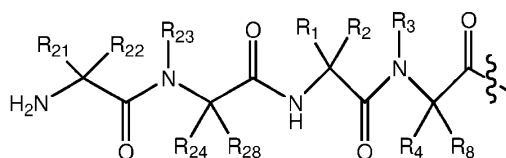
R_3 is C₁-C₆ alkyl; and

R_5 is NH₂.

44. The prodrug derivative of any one of claims 41-43 wherein said dipeptide element spacer is

-[COCH₂(OCH₂CH₂)₂-NH]-(gamma glutamic acid), and the acyl group is a C18-C20 diacid, or a C18-C20 phosphonic fatty acid.

45. The prodrug derivative of any one of claims 35-44 wherein said dipeptide element comprises a sequential dipeptide structure of



covalently linked via an amide bond to a primary amine of any one of said amylin analog of claims 1-11 or 24-30, the calcitonin selective agonist of claims 31-34 or any one of the conjugate derivatives of claims 12-23,

wherein

R_1 is (C₁-C₄ alkyl)NH₂ or (C₁-C₄ alkyl)NH₂, linked to a C16-C30 fatty acid, a C16-C30 phosphonic fatty acid, or a C16-C30 diacid, optionally via a DD1 spacer;

R_{21} is (C₁-C₄ alkyl)NH₂ or (C₁-C₄ alkyl)NH₂, linked to a C16-C30 fatty acid, a C16-C30 phosphonic fatty acid, or a C16-C30 diacid, optionally via a DD2 spacer, with the proviso that at least one of R_1 or R_2 comprises a C16-C30 fatty acid, a C16-C30 phosphonic fatty acid, or a C16-C30 diacid;

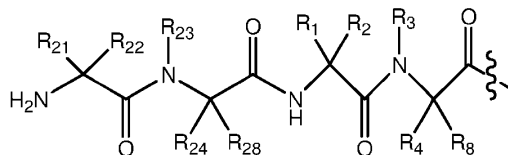
R_2 , and R_{22} are each H;

R_4 , R_8 , R_{24} and R_{28} are independently H or D;

R_3 and R_{23} are independently C₁-C₆ alkyl or CD₃, wherein said DD1 and DD2 prodrug element spacers are independently selected from the group consisting of a gamma glutamic acid, a gamma glutamic acid-gamma glutamic acid dipeptide, and a (gamma glutamic acid)_z-[COCH₂(OCH₂CH₂)_k-NH]_q-(gamma

glutamic acid)_p, wherein z is 0 or 1, k is an integer selected from the range of 2-4 and q and p are independently an integer selected from the range of 0-4.

46. The prodrug derivative of claim 45 wherein said sequential dipeptide comprises the structure:



wherein

R₁ is (C₁-C₄ alkyl)NH₂ or (C₁-C₄ alkyl)NH₂, linked to a C16-C30 phosphonic fatty acid, or a C16-C30 diacid via a DD1 spacer;

R₂₁ is (C₁-C₄ alkyl)NH₂ or (C₁-C₄ alkyl)NH₂, linked to a C16-C30 phosphonic fatty acid, or a C16-C30 diacid via a DD2 spacer, with the proviso that at least one of R₁ or R₂ comprises a C16-C30 phosphonic fatty acid, or a C16-C30 diacid;

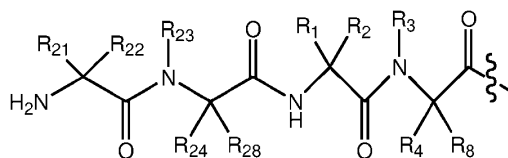
R₂, R₄, R₈, R₂₂, R₂₄ and R₂₈ are each H;

R₃ and R₂₃ are independently C₁-C₆ alkyl; and

wherein said DD1 and DD2 spacers are independently

-[COCH₂(OCH₂CH₂)_k-NH]_q-(γ glutamic acid)_p, wherein k is 2, q is 1 or 2 and p is 0 or 1.

47. The prodrug derivative of claim 46 wherein said sequential dipeptide comprises the structure:



wherein

R₁ is (C₁-C₄ alkyl)NH₂, linked to a C16-C30 phosphonic fatty acid, or a C16-C30 diacid via a DD1 spacer;

R₂₁ is (C₁-C₄ alkyl)NH₂ or (C₁-C₄ alkyl)NH₂, linked to a C16-C30 phosphonic fatty acid, or a C16-C30 diacid via a DD2 spacer;

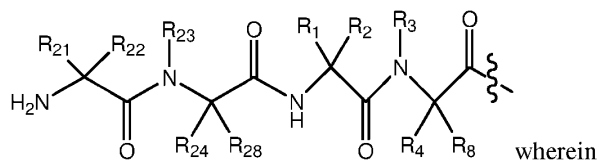
R₂, R₄, R₈, R₂₂, R₂₄ and R₂₈ are each D;

R₃ and R₂₃ are each CD₃;

wherein said DD1 and DD2 spacers are independently

-[COCH₂(OCH₂CH₂)_k-NH]_q-(gamma glutamic acid)_p, wherein k is 2, q is 1 or 2 and p is 0 or 1.

48. The prodrug derivative of any one of claims 35-47 wherein said dipeptide element comprises a sequential dipeptide structure of



R₁ comprises a (C₄ alkyl)NH₂ side chain that has been pegylated, via a DD3 spacer, with a straight chain or branched polyethylene glycol chain having a molecular weight ranging from about 20k to about 40k;

R₂₁ comprises (C₄ alkyl)NH₂ or a (C₄ alkyl)NH₂ side chain that has been pegylated, via a DD4 spacer, with a straight chain or branched polyethylene glycol chain having a molecular weight ranging from about 20k to about 40k;

R₂, and R₂₂ are independently H, or C₁-C₄ alkyl;

R₄, R₈, R₂₄ and R₂₈ are independently H, D, or C₁-C₄ alkyl;

R₃ is CD₃, C₁-C₆ alkyl, or R₃ and R₄ together with the atoms to which they are attached form a substituted or unsubstituted pyrrolidine, hydroxypyridine or piperidine ring; and

R₂₃ is CD₃, C₁-C₆ alkyl, or R₂₃ and R₂₄ together with the atoms to which they are attached form a substituted or unsubstituted pyrrolidine, hydroxypyridine or piperidine ring, wherein said DD3 and DD4 spacers are independently

-[COCH₂(OCH₂CH₂)_k-NH]_q-(alanine-triazole), wherein k is 2, and q is 1 or 2, optionally wherein k is 2, or -[COCH₂(OCH₂CH₂)_k-NH]_q-(cysteine-S-S), wherein k is 2, and q is 1 or 2, optionally wherein k is 2.

49. A pharmaceutical composition comprising the peptide of any one of claims 1 to 48 and a pharmaceutically acceptable carrier.

50. A method of reducing weight gain or inducing weight loss, comprising administering to a patient in need thereof a pharmaceutical composition of claim 49 in an amount effective to reduce weight gain or induce weight loss.

51. A method of increasing bone growth or preventing bone loss in a subject in need thereof, said method comprising administering to said subject a composition comprising the peptide of any one of claims 31-34 and a

pharmaceutically acceptable carrier in an amount effective to increase bone growth or prevent bone loss.